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Circulatory and Metabolic Changes in Experimental Intestinal Hypoperfusion

Local Metabolic Monitoring by Intestinal Microdialysis

Doctoral dissertation

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ABSTRACT

Adequacy of visceral circulation is considered important in critical illness. If intestinal blood flow is decreased or the ability of visceral tissues to extract oxygen is deranged, local and systemic inflammation may follow and thereby induce cascades towards multiple organ dysfunction. Accordingly, detection of the adequacy of gut perfusion may be important. Monitoring regional intestinal perfusion and metabolism is complicated. Therefore, research on new techniques for detection of regional perfusion and preferably methods enabling monitoring of local cellular metabolism is warranted. The objective of the present series of experiments in porcine models of hypoperfusion was 1) to develop and validate a microdialysis application for monitoring the metabolism of intestinal tissue during shock and 2) to investigate the metabolic changes together with regional circulatory changes during selective intestinal ischemia and reperfusion, cardiogenic shock and finally in distributive shock. A comparison between intestinal mucosal to arterial gradients of partial tension of carbon dioxide and gut luminal microdialysate lactate measurements was carried out. We found that 1) in vivo as well as in vitro recovery of the capillary was high (80-100%), 2) gut luminal lactate increased only slightly during nonischemic hyperlactatemia and the effect is predictable, 3) gut wall interstitial microdialysate lactate concentration follows closely arterial and venous lactate concentrations while gut luminal lactate release reflects local intestinal epithelial cellular metabolism, 4) large individual variations exist in the threshold of superior mesenteric artery blood flow that leads to increased gut luminal lactate release, 5) gut mucosal to arterial pCO_2 gap precedes the increase of gut luminal lactate release, 6) gastric mucosal to end tidal pCO_2 gap may be used as a surrogate for mucosal to arterial pCO_2 gap despite the effect of dead space ventilation on the bias and precision, 7) gut luminal lactate increases relatively late during severe cardiogenic shock, 8) intestinal mucosal epithelial injury in endotoxin shock occurs in the colon and is associated with colonic luminal lactate release. In conclusion, gut luminal lactate detection has potential for monitoring gut mucosal epithelial cellular metabolism. Increasing mucosal arterial pCO_2 gradient indicates perfusion defect in relation to metabolism while gut luminal lactate release marks the onset of dysoxia and anaerobic metabolism.

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ABBREVIATIONS

AcAc	acetoacetate
ß-OHBUT	ß-hydroxybutyrate
CI	cardiac index
CO	cardiac output
CVP	central venous pressure
DO_2	delivery of oxygen
$ETCO_2$	fraction of carbon dioxide in expired air
FiO_2	fraction of oxygen in inspired air
HR	heart rate
pHi	Intestinal mucosal pH
LPS	lipopolysaccharide
PAPm	mean pulmonary arterial pressure
PCO_2	carbon dioxide partial tension
PEEP	positive end-expiratory pressure
PO_2	oxygen partial tension
PVR	pulmonary vascular resistance
SAPm	mean arterial pressure
SMA	superior mesenteric artery
SVR	systemic vascular resistance
Qaorta	blood flow of descending thoracic aorta
Qceliac	celiac trunc blood flow
Qhep	hepatic arterial blood flow
Qporta	portal venous blood flow
Qsma	superior mesenteric artery blood flow
Ve	minute ventilation
VO_2	oxygen consumption
Vt	tidal volume

LIST OF THE ORIGINAL ARTICLES

This thesis is based on the following articles, which are referred to in the text by their Roman numerals:

I Tenhunen JJ, Kosunen H., Heino A., Tuomisto L., Alhava E., Takala J. Intestinal Luminal Microdialysis- A New Approach to Assess Mucosal Dysoxia. Anesthesiology 1999; 91: 1807-1815

II Tenhunen JJ, Jakob SM, Takala JA. Gut Luminal Lactate Release During Gradual Intestinal Ischemia. Intensive Care Medicine 2001; 27: 1916-1922

III Tenhunen JJ, Jakob SM, Ruokonen E, Takala J. Jejunal luminal microdialysate lactate in cardiac tamponade – effect of low systemic blood flow on gut mucosa. Intensive Care Medicine 2002; 28: 953-962

IV Tenhunen JJ, Uusaro A, Ruokonen E. Gastric mucosal systemic pCO_2 gradient in experimental endotoxin shock – comparison of two methods. Intensive Care Medicine 2001; 27: 1923-1930

V Tenhunen JJ, Uusaro A, Kärjä V, Oksala N, Jakob SM, Ruokonen E. Intestinal mucosal epithelial injury is associated with luminal lactate release in endotoxin shock. Submitted.

Original articles are published with the permission of the publisher. In addition unpublished data are presented

CONTENTS:

1 INTRODUCTION

Multiple organ dysfunction (MODS) with high mortality remains as one of the major clinical challenges in modern intensive care. The etiology of MODS is most probably multifactorial, but abnormalities of splanchnic perfusion are likely contributors [1,2]. Even if the controversial concept of inadequate splanchnic perfusion as a motor of MODS is accepted, clinicians are left with no reliable and feasible method to monitor intestinal perfusion and metabolism. Consequently, efforts have been made to develop methods to detect splanchnic hypoperfusion, regional metabolic changes and evaluate causal links between splanchnic organ hypoperfusion and remote organ dysfunction. Measurement of gastric mucosal arterial pCO_2 gradient by gastric tonometry is currently the only clinically feasible method to give even a surrogate estimate of the adequacy of splanchnic perfusion. Several practical and conceptual limitations of this method [3, 4, 5, 6, 7, 8] limit its value in clinical practice and therefore it is important to establish and evaluate new approaches for monitoring intestinal mucosal metabolism. Microdialysis, primarily invented for brain research [9] and recently applied also in intestinal tissues [10, 11], provides the way to measure various metabolites in tissues. Since inadequate tissue blood flow will inevitably cause distinct metabolic changes, microdialysis is a potential method also for monitoring perfusion related metabolic changes in the gastrointestinal tract.

2 REVIEW OF THE LITERATURE

2.1 Pathogenesis of multiple organ failure

This review focuses on and is limited to that part of anatomy, physiology and pathophysiology of splanchnic region, which is relevant to the perfusion abnormalities and which may be associated to inflammatory reactions and multiple organ failure.

There is probably no single event leading to or single explanation for a developing organ dysfunction in critical illness. Nevertheless, perfusion abnormalities in the splanchnic region may contribute to its pathogenesis by several mechanisms. In a most simplistic form of interactions its contribution has been described as a chain of events starting from redistribution of blood flow away from splanchnic region and consequent gut mucosal injury, translocation of bacteria and/or bacterial toxins and induction of inflammation both locally and systemically. The systemic inflammatory response could then lead to MODS. Although this original gut hypothesis of MODS is evidently a gross oversimplification, it is evident that abnormalities in splanchnic perfusion may directly cause organ dysfunction locally, modify local and regional organ function and inflammatory responses and interact with systemic circulation, remote organ function and host defense. One of the major reasons for the lack of information and controversial results is the complexity of underlying physiology and anatomy but also inadequate methods to assess it in the clinical setting.

Hypothetically, several disease states may redistribute blood flow away from splanchnic tissues [12,13,14,15] thereby leading to gut mucosal injury and concomitant mucosal barrier breakdown [16,17]. This enables bacteriae and/or bacterial toxins to invade underlying tissues [18] and either induce inflammation locally or spread into lymph ducts and vessels thereby launching systemic inflammation [19, 20]. Subsequently, sepsis or systemic inflammatory response (SIRS) leads to MODS [21,22]. If these assumptions are correct, estimating the adequacy of intestinal tissue perfusion and metabolism is important. In clinical practice, traditional systemic markers of

tissue perfusion may leave splanchnic hypoperfusion undetected [23, 24, 25] and even with stable systemic hemodynamics, splanchnic perfusion may be marginal [26]. There are also controversial studies indicative for sustained splanchnic perfusion during low systemic blood flow [27,28,29]. Similarly, even though there is some evidence for the causality between mucosal barrier breakdown, bacterial translocation and MODS [30], this is questioned by others [20].

2.2 Gut hypothesis in critical illness

2.2.1 Anatomy and physiology of visceral circulation

Differences in the anatomy and physiology of splanchnic region exist between species. In the following, we focus on human anatomy and physiology and to the species differences relevant to interpret the findings in published studies and current experiments.

Arterial supply to splanchnic vascular bed comprises of three major arteries arising from abdominal aorta: celiac trunc, superior mesenteric artery and inferior mesenteric artery. A significant degree of variation exists in anatomy of visceral circulation [31,32]. Normally (in 65-75% of cases) celiac trunc divides into three branches: splenic artery, common hepatic artery and left gastric artery. Common hepatic artery further divides into gastroduodenal, right hepatic and hepatic proper branches. Superior mesenteric artery supplies the whole of small bowel from duodenum to cecum but also the colon as far as mid-colon or halfway transverse colon. The descending colon, sigma and rectum are supplied by inferior mesenteric artery.

2.2.1.1 Gastric wall

Gastric wall is supplied by several arterial branches arising from celiac trunc, hepatic proper artery, splenic artery and occasionally from aorta. Variation in origins of the arterial supply exists in humans [33]. The main branches form the two common arcades: one towards lesser curvature and

the other towards greater curvature. Notice that in swine short gastric arterial branches are remarkably large. In pigs, the blood flow is subsequently divided into communicating arteriolar network through which arterial supply to whole thickness of gastric wall is maintained even if 1-2 arterial branches are ligated [34]. Heterogeneity of distribution in blood flow within the gastric wall occurs [35] and mucosal blood flow to crests of the mucosal folds is higher than to base of the folds [36]. There is limited data on the variability of arteriolar and capillary vessel distribution and species specific characteristics may contribute to controversial studies on gastric wall perfusion. In dogs, a single arteriolar network supplies gastric corpus, while in antrum there are two distinctive vascular networks [37]. Sympathetic and parasympathetic stimuli affect gastric wall blood flow. In canines, adrenergic stimulus induces vasoconstriction followed by vasodilation, which is more prominent in the left gastric artery [38]. Vagal stimulation induces mucosal arteriolar dilatation [39] and increases gastric blood flow [40]. Substance P and cholecystokinin increase gastric blood flow [41]. Under normal conditions, 16% of the relative blood flow is directed towards the muscle and 84% towards mucosa-submucosa [42]. Intragastric secretion of acid (H+) increases gastric mucosal blood flow under normal conditions [43].

2.2.1.2 Small intestine

The arteries within the wall of jejunum are arranged as two different plexuses: muscular and mucosal vascular network. The mesenteric arterial arcade divides into arterioles, which enter the jejunum. These arterioles branch to both sides of the mesenteric angle and supply the jejunal wall. The vessels enter the submucosa and divide, thus forming a submucosal plexus. The arterial network of the submucosa penetrates the muscularis mucosae and branches further to form mucosal capillary network [44]. Mucosal blood flow is less influenced by sympathetic vasoconstriction as opposed to muscular layer [45].

Small intestinal villous circulation is characterized by hairpin loop capillaries. Central artery rises towards the tip of the villi and runs down surrounding the

arteriole as a venous plexus. The vicinity of the arterial and venous capillaries enable oxygen exchange to occur before blood reaches the tip. This so-called oxygen counter-current exchanger may induce hypoxia to the tip of villi [46, 47]. In fact this oxygen exchanger mechanism is not limited to jejunal mucosa with hairpin structure of the capillaries, but occurs as well between larger vessels and capillaries in muscle tissue [48]. Another mechanism rendering the tips of the villi prone to hypoxia is plasma skimming [49]. The central arteriole rises from larger artery in right angle. The erythrocytes stay in the mid-stream of blood flow. The hematocrit in the central arteriole is therefore lower than in arteries of larger size. In addition, the metabolic activity of the epithelial cells in the tip of the villi may be high as suggested by Lundgren and Haglund [50].

2.2.1.3 Cecum

Microvasculature of cecum is arranged as a mesh-like network around cecal veins. From this network, arterioles extended around the circumference of cecum within submucosa thus forming an extensive submucosal plexus. This plexus supplies mucosal, muscular and serosal layers [51].

2.2.1.4 Colon

Colic branches of superior mesenteric artery supply ascending and transverse colon. Colonic microvasculature has been studied in detail in horses: Colonic arterial arcade divide into smaller branches to form a plexus, the colonic rete. Arterioles from the rete penetrate the tunica muscularis into the submucosa and form a submucosal vascular network. From the submucosal arterioles, branching occurs at right angles to supply the mucosal capillaries. These mucosal capillaries surround the colonic glands and anastomose at the luminal surface, forming a superficial luminal honeycomb-appearing vascular plexus [52]. The microvascular arrangement in the

descending colon is closely similar to the structure in ascending and transverse colon with abundant submucosal network [53].

2.2.1.5 Liver- dual blood supply

Oxygen supply to the liver is exceptional in comparison to other organs because it has dual nutritive blood supply. Approximately 60% of oxygen delivery to liver come from portal vein. The rest is supplied by hepatic artery. The blood from two different supplying vessels appears to be well mixed before reaching the parenchymal cells for metabolism [54]. The mechanism of the interplay between the two components of blood supply was elucidated by series of experiments by Lautt [55,56,57,58]. This led to understanding of dynamic interplay between the two and is called hepatic arterial buffer response. If portal venous blood flow is acutely reduced, hepatic arterial blood flow increases and thus partially restores the total oxygen delivery to liver. This is called hepatic arterial buffer response (HABR). It is mediated by adenosine, which is normally flushed away from hepatic capillary circulation. Instead, during low portal venous blood flow adenosine remains in capillaries in the so-called space of Mall and therefore induces dilatation of (hepatic) arteriolar part of intrahepatic capillaries. Thereby HABR enhances arterial oxygen delivery [59]. It is important to consider hepatic arterial buffer response and its effect on perfusion of gastric wall. Since hepatic arterial blood flow directly interacts with other arterial branches of celiac trunk it is relevant to the perfusion of gastric wall [60]. It is possible that reduced portal blood flow for any reason increases hepatic arterial blood flow by buffer mechanism. This may reduce perfusion towards other visceral organs that are supplied by arterial branches from celiac trunk. In fact, the importance of HABR or more generally the interaction between hepatic arterial and portal circulation under normal physiology is not well characterized or understood.

2.2.2 Potential association between perfusion abnormalities and multiple organ dysfunction

Multiple organ dysfunction syndrome remains a major cause of mortality and morbidity in critical illness [61, 62]. The higher the number of organs failing in the first 48 hours of critical care, the higher the mortality [63]. Even though a definite link between gut, bacterial translocation and multiple organ failure is missing, it is reasonable to assume that gut may modulate the late remote organ responses via bacterial translocation and cytokine responses [20 64]. This concept is supported by a recent clinical trial where an association between increased intestinal permeability and MODS was observed [30].

Endotoxin challenge is widely used as a model of induced inflammatory response. In short-lasting experiments with a bolus injection of endotoxin, mesenteric arterial blood flow either decreases [65] or remains at baseline. Increased mucosal permeability may occur after endotoxin injection. Endotoxin induces intestinal mucosal injury and bacterial translocation partly through increased xantine oxidase activity [66,67]. Early epithelial injury induced by endotoxin is caused by necrosis and not by apoptosis. Later during endotoxin shock apoptosis may have a role in the development of epithelial injury and this is mediated by nitric oxide (NO) [68]. Even though total splanchnic blood flow may be preserved or even increased in septic [23] and endotoxin shock [69], small intestinal tissue acidosis may occur even without mucosal hypoxia [70]. Endotoxin also induces dysregulation of normal veno-arterial response in mesenteric vascular bed and thereby leads to pooling of blood into intestinal tissues [71]. Ensuing oedema formation may further deteriorate tissue perfusion. Again, contradicting results exists and some studies suggest that the volume of total splanchnic capacitance vessels is not increased during endotoxemia [72].

2.2.3 Regional perfusion defects in shock

Circulatory shock, deranged tissue perfusion, can be classically divided into subgroups: low blood flow states associated with hypovolemia or cardiogenic shock and distributive shock like septic shock. Rather contradictory results in the literature in regard of regional blood flow and metabolic changes in experimental models of shock may be explained by differences in models, methodology and species used. In the following, regional blood flow and metabolic events are discussed as described in the literature in either low blood flow state (hemorrhagic and cardiogenic shock) or in distributive shock (endotoxin shock).

2.2.3.1 Gastric wall

In hemorrhagic shock gastric mucosal perfusion decreases and only partly recovers shortly after reperfusion [73]. In fact, mucosal perfusion may be even further decreased during reperfusion. This is at least in part mediated locally by endothelium derived substances which induce mucosal vasoconstriction and endothelium-neutrofil interaction during low blood flow and hypotension [74,75,76]. The regional blood flow changes within the gastric wall are not uniform as suggested by animal experiments [77]. Nevertheless, regional blood flow changes may actually parallel systemic flow changes during low systemic blood low as induced by cardiac tamponade [78]. This is in contrast to earlier studies where splanchnic perfusion was supposed to be preferentially reduced during low systemic blood flow [79]. Under ischemic conditions, as opposed to normal physiology, gastric acid secretion may further diminish gastric mucosal blood flow thus leading to more pronounced mucosal damage [43, 80].

E. coli bacteremia may acutely increase gastric mucosal blood flow [81]. Similarly, endotoxemia may induce gastric wall hyperemia and increased capillary perfusion. This may be dose dependent since higher dosage with ensuing systemic hypotension may actually decrease gastric mucosal perfusion [82]. Intravenously administered bacterial endotoxin

(lipopolysaccharide, LPS) may protect gastric mucosa from epithelial injury induced by intraluminal irritants [82,83]. Endotoxin stimulates bicarbonate-rich gastric fluid accumulation. This fluid accumulation may protect gastric mucosa from damage induced by acid bile [84]. There are also contradicting results with enhanced susceptibility to mucosal injury after endotoxin pretreatment [85]. These conflicting results were related to whether the animals were anesthetized or not, but also specifically to whether alkaline protective gastric fluid is removed or not [84]. In humans, gastric microvascular hypoperfusion during hyperdynamic septic shock is apparent [86].

2.2.3.2 Jejunum

Gross small intestinal perfusion through superior mesenteric artery may be maintained during hemorrhagic shock or normovolemic anemia [87, 88, 89] and redistribution of blood flow within the jejunal wall may occur [87]. These investigators found that gross intestinal wall perfusion increased but was directed preferentially towards mucosa: mucosal perfusion increased by 68% while the non-mucosal tissue compartment of the gut wall (muscular layer) increased by 32% during acute normovolemic hemodilution as compared to baseline conditions. The local increase of 32% of blood flow towards muscular and serosal layer was in proportion with increased systemic blood flow while mucosal blood flow increase was higher than expected. In hemorrhagic shock the morphological changes and high cathepsin-D, lysosome derived proteolytic enzyme activity is most pronounced in jejunum [90]. The authors suggest that cathepsin-D release within the wall of intestine signifies the breakdown of lysosomal membranes and accordingly the action of proteases damaging the intestinal tissues. Release of cathepsin-D may well be related to damage in intestinal cellular integrity in hemorrhagic shock and in part responsible for launching inflammatory response. In jejunum, hemorrhagic shock induces breakdown of mucosal barrier and is associated with diminishing cellular ATP content [91].

Due to heterogeneity in capillary blood flow and metabolic changes within intestinal wall, regional venous samples may not reflect only mucosal

oxygenation [92], but instead represent flow dependent average of the whole thickness of intestinal wall.

Small intestinal mucosal microvascular blood flow decreases without changes in regional blood flow during bacteremia in rats [93]. Others have suggested that intestinal mucosal acidosis occurs even in the absence of hypoxia or signs of deranged microcirculation [70, 94]. Ileal and cecal capillary blood flow decreases after endotoxin challenge while the rest of the intestine remains well perfused in a rat model of hyperdynamic endotoxin shock [95]. In ileum cecal ligation and following sepsis decreases capillary density in mucosa [96, 97]. Decreased capillary density in distal small bowel was detected during experimental normotensive sepsis [98]. Endotoxin induces jejunal intravascular congestion and hemorrhage as determined by increasing tissue hemoglobin concentration in rats during endotoxin challenge [99]. Endotoxin also redistributes capillary blood flow between muscularis and mucosa. In anesthetized pigs endotoxin increases jejunal, mid-small intestinal and ileal mucosal blood flow while capillary blood flow to muscular layer decreases [70]. To sum up, blood flow and metabolic changes during endotoxin or septic shock may be heterogeneous over the length of small intestine as suggested by recent experiments.

2.2.3.3 Colon

In experimental hemorrhagic shock splanchnic blood flow is heterogeneously distributed. Colonic mucosal perfusion is reduced (while jejunal mucosal perfusion may be maintained) [100] and colonic mucosal pCO_2 stagnation occurs with mucosal lesions in histological analysis [101]. In a rat model of hemorrhagic shock progressive low blood flow state induces transmural oxygen pressure gradient. Mucosal oxygen tension decreases to much higher extent as compared to serosal partial tension of oxygen [102].

In a rat model of acute endotoxemia, colonic mucosal blood flow is preserved while ileal mucosal blood flow decreases during 6 hours of observation [103]. In an experimental endotoxemia in primates mucosal blood

flow in colon is not affected [104]. In contrast Hiltebrand demonstrated that colonic blood flow decreases both in hypo- and hyperdynamic septic shock in pigs, without signs of regional hypoperfusion [105].

2.2.3.4 Hepatic arterial buffer response

During low systemic blood flow either due to cardiac tamponade [78] or hypovolemic shock [106] HABR protects liver but the response is later exhausted in cardiac tamponade [78]. In septic shock hepatic arterial buffer response is first blunted as suggested by two groups of investigators [105, 107] but restored to some extent later during endotoxin shock [107]. In effect, most studies on HABR in different patophysiological states have been conducted after changing the anatomical premises by ligating vessels in the region or by removing spleen. Therefore, our knowledge on HABR is rather limited.

2.3 Monitoring visceral perfusion and metabolism

2.3.1 Regional blood flow/perfusion

Despite hyperdynamic systemic circulation in septic/endotoxin shock gastric [86], small intestinal [68, 108, 109], and colonic [110, 111] mucosal hypoperfusion may occur according to some investigators, while others report preferentially sustained mucosal blood flow [112, 70, 113] in intestine. Monitoring regional blood flows, capillary perfusion or metabolic changes only may give wrong impression of the actual state of cellular integrity [70,105]. Furthermore, there are numerous investigations on perfusion/metabolism over visceral tissues but most of them focus on one or two locations while few have compared perfusion changes between different parts (with different arterial supply) of GI tract simultaneously [105, 114]. There have been experiments which have studied perfusion/metabolism (pCO_2 gradients) over one (gastric) visceral region, yet comparison have been made to another region (gut) neglecting the probably significant difference of the origin of arterial blood

supply (celiac trunc vs. superior mesenteric artery vs. inferior mesenteric artery). In effect, there are still confusing studies where comparison of one vascular bed (superior mesenteric arterial blood flow) is correlated to mucosal capillary perfusion changes over another visceral region (gastric mucosal near infrared spectrometry) [115].

In experimental conditions regional blood flow can be measured by perivascular ultrasonic-based methods [116,117], where the vessel of interest is prepared and visualized after which the transit time probe is applied around the vessel and fixed. Microcirculation of the tissues can be assessed by approaches such as microsphere technique, laser Doppler [118, 119] or intravital microscopy [120]. In humans total splanchnic perfusion has been evaluated by color dye dilution methods [121] or by measuring metabolic end products from liver vein [122]. Perfusion changes on regional level give only one part of information when we attempt to monitor local cellular homeostasis, while local metabolic monitoring may provide complementary inform on relevance and consequences of reduced blood flow. Due to the possibility of cellular metabolic down regulation we need to know when oxygen supply is diminished enough to induce cellular injury. In other words, we need to try to define individually the different stages of decreasing oxygen supply leading ultimately to cellular dysoxia with cellular injury [123].

2.3.2 Gastrointestinal tonometry

Boda and Murányi were the first to introduce the idea of measurement of partial tension of carbon dioxide from a body cavity (urinary bladder). They studied whether it would be possible to monitor arterial partial tension of carbon dioxide ($paCO_2$) by urinary bladder pCO_2 and thus the adequacy of systemic CO_2 removal in mechanically ventilated children with polio [124]. Paradoxically, the method was only later adopted for monitoring regional metabolism, perfusion and oxygenation. Fiddian-Green and others developed the method further in the early 80's for calculation of gastric intramucosal pH [125, 126] using modified Henderson-Hasselbach equation. The prerequisite

for the calculation was an assumption that tissue bicarbonate concentration equals arterial bicarbonate concentration. This is not necessarily true [127]. In the early 90's gastric mucosal pH was used as a surrogate marker of regional perfusion and level of regional oxygenation. Later, regional mucosal – arterial gradient of partial tension of carbon dioxide was adopted as to better reflect local perfusion.

Gastric mucosal – arterial pCO_2 gradient is considered as a surrogate for the adequacy of total splanchnic perfusion. Recently, controversial results have emerged from both basic and clinical trials. Increasing total splanchnic blood flow in either septic or postoperative patients does not necessarily decrease gastric mucosal – arterial pCO_2 gradient. It can in fact do the opposite [128, 7]. Part of the explanation may be Haldane effect: increasing tissue partial tension of oxygen or hemoglobin oxygen saturation leads to higher pCO_2 at constant tissue CO_2 content [6]. This may occur after changes in pH, temperature or regional blood flow. Another explanation for the controversy might be that the perfusion changes within intestinal region are not homogenous in various clinical situations or with different drug interventions.

Attempts have been made to determine the anaerobic threshold for mucosal arterial pCO_2 gradient [129]. Intestinal tonometry and mucosal arterial pCO_2 gradient is neither sensitive nor specific for detecting mesenteric hypoperfusion [8]. Limitations and sources of error to gastric tonometry exist: bacterial CO_2 production within gut may lead to gas supersaturation thereby invalidating the pCO_2 gradient data [130]. Blood in the GI tract also affects the CO_2 measurement [5]. In experimental models of endotoxin shock and fecal peritonitis [131] decrease in gut mucosal pHi is not necessarily associated with impaired gut mucosal oxygenation.

2.3.3 Markers of anaerobic metabolism - cytosolic and mitochondrial redox state

During progressive hypoperfusion of tissues, cells have two alternative routes to react: The cells either shift their metabolism to a lower level or conversely turn to anaerobic metabolism. Present methodology does not allow for detection of onset of anaerobic metabolism especially in a clinical setting.

Lactate is produced in excess during anaerobic glycolysis as evidenced by numerous experimental data. In septic conditions high systemic lactate concentration may be related to tissue hypoxia [132]. On the other hand, several investigators have challenged the concept of high lactate concentration as a marker of cellular hypoxia. Lactate release from the cells is not necessarily caused by hypoxia as exemplified by Luchette and colleagues but rather by increased activity of Na-K-ATP-ase [133, 134, 135, 136].

Connett and colleagues suggested in their review [123] that it is important to redefine the terminology concerning inadequate oxygen supply to tissues in relation to cellular oxygen demand: 1) *hypoxia* should be considered only to signify lower than normal partial tension of oxygen in inspired air. 2) *hypoxemia* denotes only lower than normal partial tension of oxygen in arterial blood. 3) *adapted cellular hypoxia* should refer to lower than normal intracellular partial tension of oxygen with maintained O_2 and ATP flux due to adaptive changes in reduction-oxidation and phosphorylation state. Finally, 4) *dysoxia* can be defined as O_2-limited cytochrome turnover.

Schlichtig and colleagues studied whole organ (liver) oxygen supply-demand relation in a hemorrhagic shock model in dogs [137]. They chose to use hepatic venous ß-OH-butyrate/acetoacetate (ketonebody) ratio as a marker of mitochondrial redox state. The rationale behind this is that if intracellular oxygen tension is decreased while oxygen demand (metabolic rate) is maintained mitochondrial NAD is bound to become reduced. Thereby, mitochondrial redox state reflects oxygen supply in relation to oxygen demand. If, during decreasing oxygen supply, oxygen consumption decreases

(supply dependence) and concomitant mitochondrial reduction of NAD occurs, then supply dependence represents dysoxia.

Reduction of NAD to NADH and thereby NADH/NAD ratio is a measure of mitochondrial redox state. ß-OH-butyrate and acetoacetate equilibrates with mitochondrial NADH/NAD because ß-OH-butyric acid hydroxygenase is located in mitochondrial christae and is coupled with the reduction processes within as described first by Lehninger [138]. Furthermore, ßOH-butyrate/acetoacetate in hepatic venous samples correlates with hepatic cellular cytochrome oxidase reduction [139,140]. Schlichtig and colleagues found that oxygen supply dependency in intact liver is paralleled with mitochondrial reduction and increasing ketonebody ratio [137]. Dishart further defined dysoxia as: decreasing ATP flux (decreasing VO_2) in parallel with decreasing cellular O_2 availability (decreasing DO_2) accompanied by increasing ATP demand [141].

2.4 Microdialysis

Microdialysis is a method which allows metabolic monitoring of one organ or tissue. A semipermeable membrane capillary is introduced into tissue and a continuous flow of dialysate fluid is pumped through the capillary. Different molecules and substances diffuse into the dialysate while the dialysate passes through the semipermeable part of the capillary. The concentration of different substances in the dialysate, which is collected as a sample, reflects the constitution of extracellular fluid. The idea of measuring the constituents of extracellular water by introducing a hollow dialysate membrane capillary into the CNS tissue of experimental animals was first described by Bito and colleagues [142] but also by Delgado and colleagues [143]. Ungerstedt and Pycock developed the method further [9]. During the first years the method was mainly used in brain research but later it has been applied to human studies and also in clinical practice in a variety of tissues. The method has been used in excess for detecting tissue metabolism, neuronal signal

transmission, drug delivery studies and pharmacokinetics [144, 145, 146, 147, 148].

2.4.1 Intestinal Microdialysis

By the year 1993 one group of investigators had applied intestinal wall microdialysis for detection of 5-HT after cisplatin exposure [149]. Thereafter we [10] and others [11, 150] reported intestinal wall microdialysate histamine, hypoxantine and lactate changes in experimental intestinal ischemia/reperfusion and endotoxin shock. Earlier Ljungdahl and colleagues had studied gut wall lactate production by using segmental occlusion technique. In that method a short segment of intestine is occluded with a double balloon catheter and the occluded space between the balloons is perfused for a period of time to allow the molecules and metabolites to stabilize in the perfusate. A sample is then aspirated from the perfusate to allow detection of for example lactate [151]. One of the benefits of microdialysis as compared to segmental occlusion technique is that long period of stabilization for the sample is not needed. Therefore the time resolution of the samples describing local metabolic changes may be higher. In addition, microdialysate samples are pure in terms of enzymatic activity. Intestinal luminal microdialysis has now been used by Solligard and colleagues in an experimental model of aortic cross clamping. They found that luminal release of lactate is associated with intestinal mucosal barrier breakdown [152]. Intestinal wall microdialysis was used for interstitial and luminal histamine detection in hemorrhagic shock [153]. Intestinal wall microdialysate lactate, pyruvate, glucose and glycerol concentrations have been used to detect intestinal cellular metabolism during endotoxemia and the effects of prostacyclin in cats [154].

3 AIMS OF THE PRESENT STUDY

The objective of the present series of experiments was to validate a new microdialysis capillary for detection of intestinal mucosal hypoperfusion and ischemia/dysoxia and describe systemic and regional circulatory and metabolic changes during shock in two different models of shock.

Specifically, we aimed:

- To test the hypothesis that microdialysis can be used to detect intestinal mucosal lactate release in:
  - Regional, selective mesenteric ischemia/reperfusion (total or partial occlusion of superior mesenteric artery)
  - Selective gradual occlusive mesenteric ischemia (stepwise occlusion of superior mesenteric artery)
- To assess gut mucosal lactate metabolism in two different experimental models of shock:
  - Cardiogenic shock with low systemic blood flow (progressive cardiac tamponade)
  - Distributive shock (prolonged endotoxin shock)

4 MATERIALS AND METHODS

The institutional animal use and care committee in the University of Kuopio approved the study protocol of each experiment. Original publication I consisted of 24 animals divided into four groups, publication II of 14 animals divided into two groups, publication III of 12 animals divided into two groups, and publications IV and V from the same experimental study with 14 animals divided into two groups. Detailed description of the relevant methods is written in each original investigation. General methodological aspects, however, are provided here.

4.1 Laboratory animals

We used Finnish female pigs of random breed in our experiments. The animals were transported to National Animal Laboratory Centre 4-5 days before each experiment to let them stabilize. An overnight fasting with free access to water was accomplished except in endotoxin shock model, where 72 hours of fasting with emptying of GI tract was applied.

4.1.1 Mode of anesthesia

The animals were premedicated with atropine 0.05 mg kg-1 and azaperone 8 mg kg-1 (and ketamine hydrochloride 200-250 mg, endotoxin model) intramuscularly. Thereafter an ear vein was cannulated for administration of sodium thiopentone 5-15 mg kg-1 to induce anesthesia. In experiments I-II anesthesia was maintained with continuous infusion of thiopentone 5mg/kg/h. In experiments III-V infusion of fentanyl was added (30 µg/kg/h during surgical manipulation and 5 µg/kg/h thereafter). Neuromuscular blocking agents were not used.

4.1.2 Mechanical ventilation

In original publications I-III the trachea was intubated while in publications IV-V a tracheotomy was performed ensuring rapid airway access. The lungs were mechanically ventilated (Servo Ventilator 900, Siemens Elema AB,

Solna, Sweden) with a constant tidal volume of 10 ml/kg and FiO_2 of 0.30 and volume controlled mode without applying PEEP in experiments I-II. In experiments III-V the lung was ventilated using a volume-controlled mode (Servo 900E, Siemens Elema, Sweden) with 10-15 ml kg-1 tidal volume to achieve normocapnia. Arterial partial pressure of CO_2 ($PaCO_2$) was kept between 4,5-5,5 kPa (34-41 mmHg) and the minute volume (V_E) of the ventilator was adjusted accordingly. Fraction of oxygen in the inspiratory gas (FiO_2) was adjusted to keep arterial partial pressure of O_2 >13,3 kPa (100 mmHg). Positive end expiratory pressure of 5 cmH_2O was used throughout the experiment. In publications IV and V serial static pressure measurements were carried out to rule out the possibility of increasing static inspiratory pressure and intrinsic PEEP.

4.1.3 Fluid management

We infused saline 0.9% 5 ml/kg/h as maintenance fluid therapy during each experiment. In experiments III-V we targeted in normovolemia during the experiment as estimated by serial pulmonary arterial occlusion pressure (PAOP) measurements. Infusions of Ringer's acetate (Ringersteril, Orion-Medipolar, Oulu, Finland) and hydroxyethyl starch (Plasmafusin, Kabi-Pharmacia, Uppsala, Sweden) in ratio of 1:1 were adjusted accordingly. In experiments IV-V infusion of glucose (50%) was used aiming at maintenance of normoglycemia (blood glucose 5-7 mmol/L).

4.1.4 Body temperature

In experiments I-II the body temperature was maintained at baseline level and in III-V with longer stabilization period normothermia (38-89 C) was achieved and maintained. We used thermistor controlled operation table heater, warmed fluids and heat reflector lamp. Active cooling was used as needed.

4.1.5 Animal preparation

In common to all experiments, either carotid artery or femoral artery was cannulated (single lumen catheter, Arrow, Arrow International Inc., Reading, PA, USA) for blood pressure measurements and blood sampling. Internal jugular vein was used for access to pulmonary artery catheterization (Arrow International Inc., Reading, PA, USA) and subclavian vein for hepatic venous catheterization (Cordis, Cordis corp., USA). Midline laparotomy was performed and vessels prepared for perivascular transit time ultrasonic flow measurement (Transonic Systems Inc., Ithaca, NY, USA). Probes of proper size were applied around vessels. The flow probes are calibrated by the manufacturer. In addition, the calibration of the flow probes was confirmed in our laboratory [78]. The transit time ultrasonic volume flowmeter has been demonstrated to provide adequate measures of arterial and venous flows in experimental animals if carefully positioned and aligned with respect to the vessel [155 156]. Signals from the flowmeters (T206 and T106, Transonic) were recorded on a computer program for further analysis (Dataq instruments Windaq 1.60, Dataq instruments Inc., Akron, OH, USA). At the end of each experiment the animals were killed with intra-venous magnesium sulfate while they were still anesthetized.

4.2 Laboratory analyses

4.2.1 Lactate

We used enzymatic lactate oxidase method with polarographic detection for both microdialysate and plasma lactate measurement (YSI 2300 Stat Plus, Yellow Springs Instruments Co. Inc., Yellow Springs, OH). In our laboratory, the interassay coefficient of variations (CV%:s) for mean concentrations of 5.2 mmol/L and 15.1 mmol/L were 2.7% (n=27) and 2.0% (n=27), respectively. The intra-assay CV%:s for mean lactate concentrations of 0,8 mmol/L (n=10), 3,3 mmol/L (n=10) and 7,9 mmol/L (n=19) were 1.3%, 0.9% and 0.6%.

4.2.2 Pyruvate

We measured whole blood pyruvate enzymatically (SIGMAS UV-706 kit, Sigma Diagnostics, St. Louis, MO, USA) with spectrophotometric detection (SHIMADZU CL-750, Shimadzu Corporation, Kyoto, Japan). The pyruvate measurements were performed daily. Intra-assay CV% at mean concentration of 101 µmol/L was 4.4%. Interassay CV% for mean pyruvate concentrations of 100 µmol/L and 200 µmol/L were 4.3% and 2.6% (n=31).

4.2.3 ß-hydroxybutyrate and acetoacetate

Plasma acetoacetate and ß-hydroxybutyrate (OHBUT) concentrations were determined with enzymatic method (hydroxybutyratehydrogenase, Boehringer Mannheim, Germany). Briefly: In acetoacetate detection OHBUT-dehydrogenase catalyzes the reaction: acetoacetate+NADH+H+ $\rightarrow$ OHBUT + NAD. The acetoacetate concentration is detemined cinetically by detecting the reduction of NADH absorbance at 340 nM wavelength. ß-OHBUT is determined with reversed reaction. The intra-assay coefficients of variation at mean concentrations of 0.24 mmol/L and 1.0 mmol/L of acetoacetate were 10.9% and 2.9%, (n=10). The interassay CV% at the same concentrations were 8.9% and 4.5% (n=31). Intra-assay CV%:s for ß-hydroxybutyrate at 0.22 mmol/L and 3.88 mmol/L were 6.9% and 1.4%, (n=10). Interassay CV%:s were 6.5% and 2.0% (n=31).

4.2.4 Blood gas analysis

Arterial and regional blood gases were analyzed within 5 minutes after carefully eliminating any gas from the syringes (ABL 520, Radiometer, Copenhagen, Denmark). Temperature-corrected values were used.

4.2.5 Hemoglobin, oxyhemoglobin and oxygen transport

Hemoglobin concentration and hemoglobin oxygen saturation were measured within 5 minutes of sampling with an analyzer in pig mode

(Hemoxymeter OSM3, Radiometer, Copenhagen, Denmark). The calculations were performed as follows: Blood oxygen content: cO_2 (ml/L) = (Hb (g/L)*SO_2*1.34)+(pO_2(kPa)*0.2325).

4.2.6 Endotoxin

Plasma endotoxin concentrations were determined by Limulus Amebocyte Lysate assay (LAL, Charles RiverEndosafe, Charleston, SC, USA) with chromogenic quantitation (Coatest endotoxin, Chromogenix AB, Mölndal, Sweden).

4.2.7 Interleukin-1ß and -6

Arterial serum cytokine concentrations (IL-1ß and IL-6) were determined with porcine immunoassay for IL-1ß [intra-assay CV for mean of 323 pg/ml (n=22) was 5.8% and interassay CV for mean of 355 pg/ ml (n=6) was 7.5%] and IL-6 [intra-assay CV was 4.1% for mean of 279 pg/ml (n=22) and interassay CV was 5.6% for mean of 327 pg/ml (n=6)] (Quantikine P, R&D Systems, Inc. Minneapolis, MN, USA).

4.2.8 Histology

Tissue samples for histology were taken from colon and jejunum before closing the laparotomy and at the end of the experiment. Biopsies were formalin-fixed and paraffin embedded. Sections were cut at 4 μm and stained with hematoxylin and eosin for routine histopathological assessment. All sections were graded for mucosal injury in a blind fashion by a pathologist without having any data on experimental setting. The sections were first graded according to Chiu´s classification [157] and further evaluated with special emphasis on changes in surface epithelium, villi and crypts. The amount of inflammatory cells was also evaluated. The histological findings in epithelial cells were separately graded from 0 to 3, where 0 indicated no histological abnormality and 1 slight, 2 moderate and 3 strong histopathological abnormality.

4.3 Microdialysis

4.3.1 Capillary and sample collection

The microdialysis capillary was manufactured in our laboratory. Capillary membrane of polysulfone with 60000 Dalton of pore size was used (Fresenius, Fresenius AG, Bad Homburg, Germany). The inner diameter of the capillary was 200 μm. The length of the semipermeable part of the capillary was 2 cm. The outflow tubing was of 0.134 mm inner diameter (PTFE transparent, BOC Ohmeda, Espoo, Finland) with a total length of 100 cm from the capillary to the sample tube. The time delay from the capillary to the sample tube was 7 minutes. The sampling was adjusted according to interventions. The microdialysate (Ringer's acetate, Ringersteril, Orion-Medipolar, Oulu, Finland) flow rate was adjusted to 2 μL/minute with microdialysis pumps (Carnagie Medicine, Stockholm, Sweden). Each sample was collected into ice cooled polypropylene tubes.

4.3.2 Microdialysis within artery, vein and gut wall

Microdialysis capillaries were inserted into the mesenteric vein and artery (mesenteric artery only in experiment 1): an 18-gauge needle was guided in and out of the vessel and the capillary was pulled inside the vessel. In the jejunal wall both the serosal (deeper layer) and the mucosal side (superficial layer) were assessed. In the serosal approach, an 18-gauge needle was visually guided under the serosa and guided out from the tissue after which the capillary was pulled into tissue. A blunt headed metal instrument was first pushed through the jejunal wall to get the mucosal approach. The tip was mounted against the mucosa and with gentle compression against the wall the instrument was guided inside the tissue for the length of 2.5-3 cm. The tip was then pushed out of the wall and the microdialysis capillary was pulled inside the channel. A needle was pushed in and out of the jejunal wall and a capillary guided inside the lumen of jejunum for the luminal approach. All the capillaries were fixed with sutures. A gastrointestinal tonometer (Tonometrics Inc., Hopkinton, MA, USA) was placed into the jejunum through an

antimesenteric incision 80 cm distally from duodenum. The placement of the microdialysis capillaries in the jejunal wall was confirmed both macroscopically after each experiment and by histologic samples.

4.3.3 Intestinal luminal microdialysis

The in- and outflow tubing for microdialysate sampling was inserted inside the tonometer catheter and the microdialysis capillaries were fixed on the surface of the tonometer balloon. After we inserted the tonometer into intestinal lumen, the balloon compressed the capillaries against the mucosa thus allowing monitoring mucosal metabolism (Figure 1).
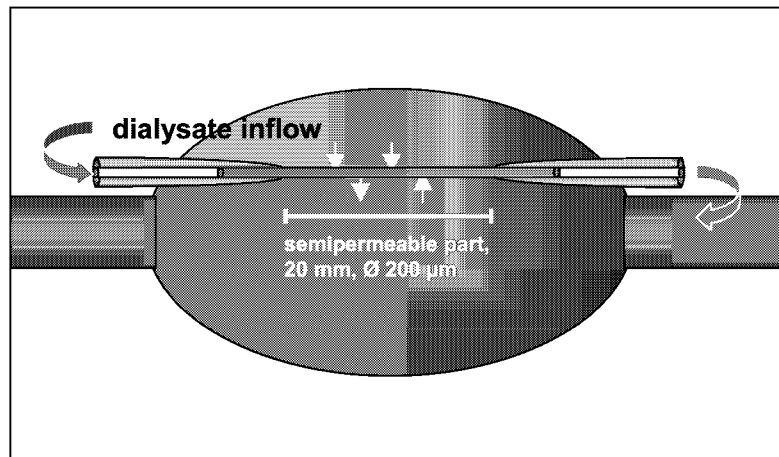
Figure 1. The structure of the microdialysis catheter – tonometer combination.

4.3.4 In vivo and in vitro recovery

In the original experiment number I, a capillary was inserted in mesenteric artery. One 30-minute baseline microdialysate lactate sample was collected. A continuous intravenous infusion of sodium lactate at 4 mmol/kg/h was started after a priming dose of 6 mmol/kg. The infusion rate was adjusted according to whole blood lactate measurements taken every 5 minutes in order to obtain a steady state for two 90 minute steps with target lactate concentrations of 5 mmol/L and 10 mmol/L. The whole blood lactate samples were drawn and injected immediately into 2 ml tubes (Trisodium-citrate with citric acid and NaF-EDTA, Venoject, Terumo Europe N.V., Leuven , Belgium).

The whole blood lactate was measured within five minutes from sampling (YSI 2300 Stat Plus, Yellow Springs Instruments Co. Inc., Yellow Springs, OH). Arterial microdialysis lactate was collected over 30-minute intervals during each 90-minute step. Thus 3 consecutive microdialysate lactate concentrations were obtained during each step. These concentrations were compared to mean arterial whole blood lactate concentration over the same time period. The agreement between arterial microdialysate lactate and the mean arterial whole blood lactate corresponding to the 30 minute microdialysis lactate sampling period was evaluated according to Bland and Altman [158]. The mean difference of the two methods represents the bias of the microdialysis lactate measurement. The limits of agreement were calculated as ±2 SD of the difference between the two methods.

In vitro recovery and reproducibility was tested for six capillaries in two different lactate solutions of 0.74 mmol/L and 4.2 mmol/L. The solution was warmed to 38 degrees of Centigrade.

4.4 Intestinal tonometry

In the experiment I jejunal mucosal partial pressure of CO_2 (PCO_2) was measured using saline tonometry (Tonometrics Inc., Hopkinton, MA, USA) and 30 minutes of equilibration. The values were corrected for the equilibration time. The PCO_2 of the saline sample and the corresponding arterial blood PCO_2 were measured using a blood gas analyzer (ABL 520 radiometer, Copenhagen, Denmark) and the gut mucosal - arterial pCO_2 gradient calculated.

In all other experiments we measured intestinal mucosal partial pressure of carbon dioxide with a semiautomatic gas analyzer [159] (Tonocap®, Datex-Ohmeda, Helsinki, Finland) every 10 minutes throughout the experiments. The device was calibrated every 2 months according to the guidelines of the manufacturer. We did not use H_2-blockers.

In experiment V end tidal PCO_2 ($EtCO_2$) was recorded continuously (CS3, Datex-Ohmeda, Helsinki, Finland). Tonometric-EtCO2 gradient ($DPCO_2gas$) was calculated every 10 minutes. Tonometric-arterial PCO_2 gradient (D(t-

a)PCO$_2$) was calculated at baseline, after 30 minutes of endotoxin infusion and every 2nd hour thereafter.

4.5 Experimental animal models

4.5.1 Lactate clamp

The details of lactate clamp were given in chapter 4.3.4. We used lactate clamp not only to estimate in vivo recovery of the capillary but also to test the effect of systemic hyperlactatemia on both intestinal wall and gut luminal microdialysate lactate concentrations. Upper normal limit of gut luminal lactate in relation to arterial hyperlactatemia was defined.

The experimental setting in lactate clamp is illustrated figure 2., where the baseline and the two different steps of lactate infusions (arterial blood lactate concentrations) are visualized. Corresponding intra-arterial dialysate lactate concentrations are presented. These two were compared in Bland-Altman analysis to test the precision of microdialysate lactate to reflect 'true' arterial lactate concentration.
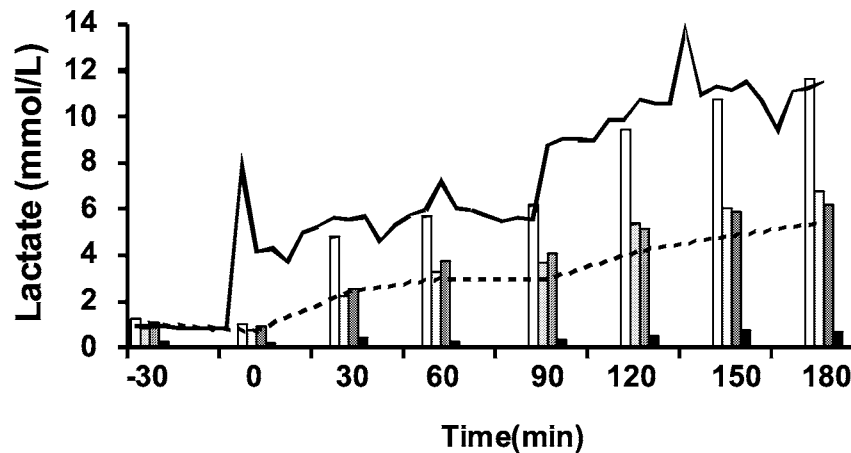
Figure 2. Arterial whole blood lactate (continuous black line) as measured every 5 minutes. Corresponding mesenteric venous (dashed line) and intra-arterial microdialysate lactate (white columns) every 30 minutes, intestinal wall microdialysate lactate (light and dark grey columns) and jejunal luminal lactate (black columns) in non-ischemic systemic hyperlactatemia (lactate clamp).

4.5.2 Selective intestinal ischemia and reperfusion

4.5.2.1 Total and partial occlusion of superior mesenteric artery

During the 90 minutes of stabilization one baseline sampling of microdialysate lactate was performed over the last 30 minutes. After the stabilization animals were randomized into 3 groups: total occlusion of superior mesenteric artery (n=6), partial occlusion of superior mesenteric artery (3 ml/kg/min flow) (n=6) and control (n=6). We used sealed opaque envelopes for randomization. The gut ischemia consisted of 90 minutes of total or partial superior mesenteric artery occlusion followed by 60 minutes of reperfusion. Total occlusion of the superior mesenteric artery was performed immediately after the baseline samples and measurements. Partial occlusion was achieved within 5 minutes by adjusting the flow. In the control group the occluder was kept in place for the same period of time without occluding the superior mesenteric artery.

4.5.2.2 Gradual decrease of superior mesenteric artery flow

The surgical preparation was followed by 90 min of stabilization after which baseline measurements were performed. During the following 60 min superior mesenteric artery flow (Qsma) was reduced in 15 minute steps by 22%, 44%, 66% and 88% from the baseline by tightening the superior mesenteric artery occluder. The blood flow was readjusted if necessary in order to keep it constant for the subsequent 15 minutes. Tonometric pCO_2 and gut luminal lactate were measured during each step. After the gradual stepwise reduction superior mesenteric artery flow was kept constant at 88% reduced level for 60 minutes. Four 15-minute microdialysate samples were collected during stable low flow. Blood samples were taken from carotid and pulmonary arteries, and from liver, portal and mesenteric veins for blood gases and plasma lactate at baseline, after stepwise reduction of superior mesenteric artery flow and after 60 minutes of constant low flow. In the animals without gut ischemia the same measurements were carried out.

4.5.3 Cardiogenic shock – cardiac tamponade

Following baseline measurements and samples, cardiac output was reduced in 2 steps lasting 60 minutes each by instillation of warmed hydroxyethyl starch into the pericardial space. Target aortic blood flows were 50 and 30 ml/kg/min. During the study period, jejunal luminal microdialysate lactate was collected every 30 minutes. Blood samples from the carotid artery, pulmonary artery, portal, mesenteric and liver vein were drawn at the end of the stabilization period and every 30 minutes thereafter until the end of the experiment for the measurement of blood gases, lactate, pyruvate, ß-hydroxybutyrate, acetoacetate and hemoglobin concentrations. In the control animals, the corresponding measurements were carried out without applying low systemic blood flow.

4.5.4 Distributive shock - endotoxin shock

The animals were allowed to stabilize for 8-10 hours after the surgery. Thereafter, the animals were randomly allocated into two groups: ETX (n=7) with E. coli endotoxin infusion (lipopolysaccharide B 0111:B4, Difco Laboratories, Detroit, MI, USA) or Control (n=7) which served as control group. We used sealed opaque envelopes for randomization.

After the baseline measurements and blood samples, infusion of E. coli endotoxin (20 µg ml-1 in 5% glucose, ETX) was started at a rate of 1.0 µg kg-1 h-1. After 2-4 hours, the infusion rate was increased stepwise to induce systemic hypotension. However, mean pulmonary arterial pressure was not allowed over 40 mmHg.

4.6 Calculations and statistical analysis

4.6.1 Lactate kinetics

Regional lactate kinetics (gastric, jejunal and colonic) were calculated by using arterial and regional lactate concentrations and regional blood flow data.

- (regional venous concentration-arterial concentration) * regional blood flow

Hepatic lactate and pyruvate exchange was calculated as

- Hepatic influx – hepatic efflux
- Hepatic influx = Q_{porta} * (portal venous concentration) + ($Q_{hepatic\ arterial}$) * (arterial concentration)
- Hepatic efflux = (Q_{porta} + $Q_{hepatic\ arterial}$) * (hepatic venous concentration)

4.6.2 Oxygen transport

Systemic and regional oxygen transport variables were calculated as follows:

- Arterial blood oxygen content CaO_2 (ml/L) = (Hb (g/L)*SaO_2*1.34)+(paO_2(kPa)*0.2325)

- Systemic oxygen delivery DO_2 (ml/kg/min)= CI*(CaO_2)

Regional oxygen delivery was estimated:

- Regional venous oxygen content (ml/L) = (Hb_{venous} * $S_{venous}O_2$*1.34)+($p_{venous}O_2$*0.2325)
- Hepatic oxygen delivery $D_{liver}O_2$ (ml/kg/min) = [$Q_{hepatic}$ * (CaO_2)]+[Q_{portal}*($C_{porta}O_2$)]
- Mesenteric O_2 delivery $D_{mesenteric}O_2$ = Q_{sma}*CaO_2

Regional oxygen consumption was calculated:

- Hepatic oxygen consumption = $V_{liver}O_2$ = $D_{liver}O_2$-[(Q_{portal}+$Q_{hepatic}$)*$C_{hepatic\ vein}O_2$]
- Jejunal wall O_2 consumption $V_{jejunal}O_2$ = (CaO_2-$C_{mesenteric\ vein}O_2$)*Q_{sma}

4.6.3 Carbon dioxide content and production

In the experiments III-V regional venoarterial CO_2 content gradients were calculated according to iterative procedure first described by Giovannini et al [160] and later used and modified by Jakob et al [6]. Regional CO_2 production was calculated by multiplying the veno-arterial CO_2 content gradient by the corresponding regional blood flow.

4.6.4 Statistics

In original article I the data is presented as median and range and in other articles as median and interquartile range. This was considered relevant since the normal distribution of the data cannot be assessed with such limited number of animals per experiment. For the same reason, we used nonparametric tests to evaluate statistical significance. When considered appropriate, Mann-Whitney U or Kruskall Wallis tests were used. We tested within the group changes over time by Friedman's nonparametric test for repeated measurements. If needed, the first significant change from baseline was located by Wilcoxon's signed rank test. Statistical significance was considered at $p < 0.05$.

5   RESULTS

5.1   Microdialysis in arterial hyperlactatemia

The characteristics of the lactate clamp are presented in the table 1. The two steps of the clamp were fairly constant as indicated by low coefficient of variation of the sequential blood lactate concentration measurements (Table 1).

Table 1. Characteristics of the lactate clamp

	Baseline			p
Time (min)	0-30	61-90	151-180	
Lactate infusion (mmol/kg/h)				
	-	3.12(3.09-3.21)	5.74(5.56-5.87)	
CV% of arterial whole blood lactate (%)				
	5.5(2.4-15.4)	3.7(1.4-14.7)	3.5(2.0-14.7)	
Arterial whole blood lactate (mmol/L)				
	1.05(0.68-1.82)	5.18(4.40-6.00)	11.2(10.1-12.3)	
Arterial microdialysis lactate (mmol/L)				
	1.20(0.76-2.01)	5.69(4.68-6.15)	11.6(10.3-12.6)	
Luminal microdialysis lactate (mmol/L)				
	0.10(0.06-0.28)	0.50(0.15-1.18)	0.86(0.35-2.05)	<0.0001
Gut wall lactate, superficial (mmol/L)				
	1.02(0.64-1.94)	2.77(1.96-4.08)	4.30(3.36-6.83)	<0.0001
Gut wall lactate, deeper (mmol/L)				
	0.81(0.71-1.94)	3.80(2.36-4.21)	6.35(4.38-6.91)	<0.0001
Mesenteric venous lactate (mmol/L)				
	0.81(0.61-1.71)	2.98(2.51-4.44)	5.69(5.14-10.10)	<0.0001
Tonometric-arterial pCO2 gap (mmHg)				
	5.4(4.8-6.0)	9.6(6.8-10.7)	11.6(9.1-10.0)	<0.0001

CV%, coefficient of variation calculated from six consecutive individual whole blood lactate concentrations. Statistical significance of changes within the group was tested with Friedmans test (the last column).

5.1.1 In vitro and in vivo recovery of the capillary

Recovery of a capillary refers to how many percents the concentration in microdialysate as compared to the real concentration in the tissue or surrounding media is. In vitro recovery was 78% at mean concentration of 0.74 mmol/L (n=6) with CV of 1.9% between the capillaries and 82% at 4.2 mmol/L (n=5) with CV of 2.6%. High level of agreement was observed between the arterial blood microdialysate lactate and the arterial whole blood lactate during the lactate clamp. The bias was 0.28 mmol/L (95% limits of agreement; -0.63-1.20 mmol/L, figure 3). The arterial microdialysate lactate closely tracked the arterial whole blood lactate concentration during the whole experiment indicating high recovery (table 1).
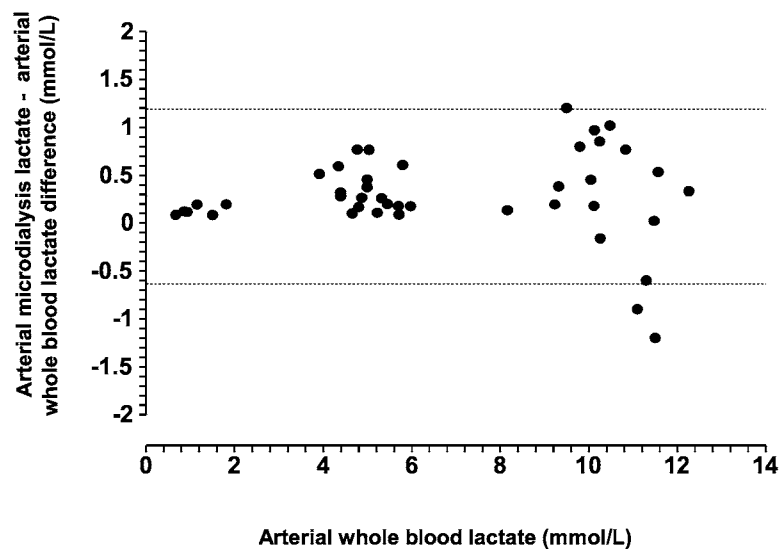
Figure 3. Bland-Altman analysis of arterial whole blood lactate measurements (mean of six consecutive 5-minute samples) as compared to arterial-microdialysate lactate difference. Individual data points (black circles) during the baseline and the two steps of lactate clamp. Bias (Continuous black line) and precision (dashed line) are presented

5.1.2 Upper normal limit of gut luminal lactate

Arterial hyperlactatemia without intestinal ischemia increases jejunal luminal lactate to some extent. Therefore the effect of arterial lactate on gut luminal lactate was estimated. The upper normal limit of gut luminal lactate in relation to arterial hyperlactatemia during nonischemic systemic hyperlactatemia is visualized in Figure 4. This approach is used subsequently in publication III (for details of correction see original publication III).

$$y = 0.1733x + 0.1769$$
$$R^2 = 0.988$$
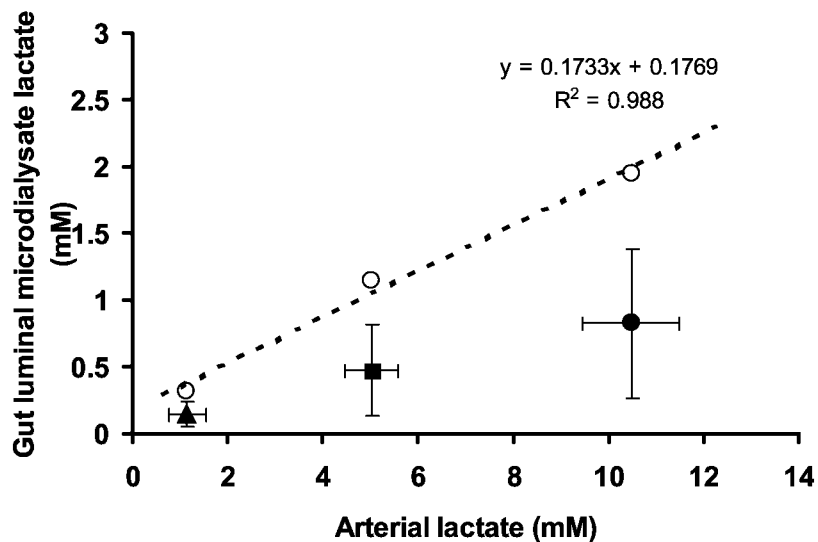
Figure 4. The effect of arterial hyperlactatemia on jejunal luminal lactate was tested by plotting the arterial lactate concentrations against the corresponding jejunal luminal microdialysate lactate concentrations during baseline conditions (triangle) and the two steps of lactate clamp (quadrangle and circle). Mean±SD with mean+2SD (open circles) were drawn and a linear trend line by least square method was determined through 2SD values (dashed line) to describe the upper normal limit of gut luminal lactate in relation to arterial lactate.

5.2 Occlusive gut ischemia and endotoxin shock

5.2.1 Total and partial occlusion of superior mesenteric artery

5.2.1.1 Systemic and regional blood flows

Systemic blood flow remained constant in both total and partial occlusion of superior mesenteric artery and after the release of the occlusion. Cardiac index was 104(72-133) ml/kg/min and 102(81-158) ml/kg/min at baseline, 112(82-250) and 95(68-193) ml/kg/min after 90 minutes of ischemia and 98(53-148) ml/kg/min and 114(87-132) ml/kg/min after 60 minutes of reperfusion in total and partial occlusion groups respectively.

Superior arterial blood flow decreased form basal 15(7-27) ml/kg/min and 15(10-27) ml/kg/min to zero and 3(2-3) ml/kg/min after 30 minutes of ischemia and increased to 20(9-40) ml/kg/min and 16(12-26) ml/kg/min after 60 minutes of reperfusion.

5.2.1.2 Gut luminal lactate and jejunal mucosal arterial pCO_2 gradient

Jejunal luminal microdialysate lactate increased from the baseline levels to over 10-fold after 30 minutes of total occlusion of superior mesenteric artery and over 5-fold after 90 minutes of partial occlusion (table 2, Figure 5). Both partial and total occlusion of superior mesenteric artery induced parallel increase in jejunal mucosal arterial pCO_2 gradient and jejunal luminal lactate. During reperfusion phase however, mucosal to arterial pCO_2 gradient decreased rapidly while gut luminal lactate remained high or even increased further (Figure 5).
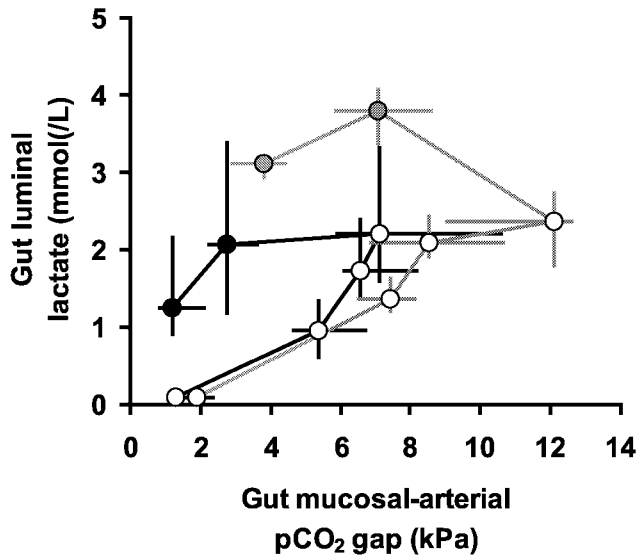
Figure 5. Gut mucosal to arterial pCO_2 gradient increase was accompanied by gut luminal lactate release as compared to baseline both in partial (open black circles and black line) and in total occlusion (open gray circles and gray line) of superior mesenteric artery. During reperfusion, pCO_2 gradient decreased shortly after release of the occlusion (filled circles) while gut luminal lactate remained high after partial occlusion (filled black circles) or even increased after total occlusion (closed gray circles). Data is presented as median and inter quartile range.

5.2.1.3 Metabolic changes

Veno-arterial lactate gradient over jejunal wall increased from 0.1(0.1-0.2) mmol/L and from 0.1(0.0-0.3) mmol/L to 4.3(0.2-7.5) mmol/L and 1.9(0.4-5.9) mmol/L after 90 minutes of total and partial occlusion of superior mesenteric artery. Arterial plasma lactate concentrations increased from low baseline concentrations to 2-3 times higher after 60 minutes of ischemia in total and partial occlusion, respectively. In jejunal wall microdialysis, we found that interstitial lactate concentrations were higher closer to mucosa as compared to interstitial concentrations closer to muscular layer (Table 2). The structure separating the capillaries closer to serosa and closer to mucosa was verified by histology (Figure 6).
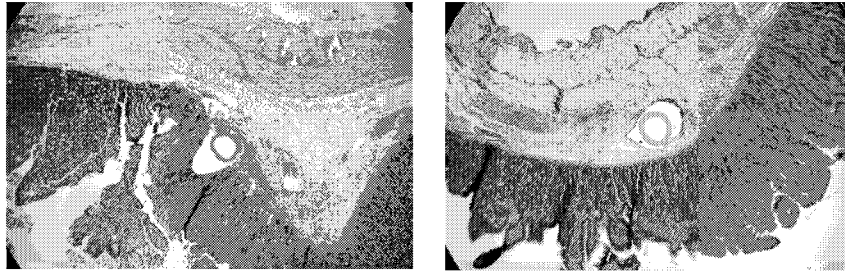
Figure 6. Representative pictures showing the localisation of microdialysis capillary (cross section, 200 μm diameter) in superficial (closer to mucosa, left) and deeper layer (closer to muscular layer, right) of mucosa as separated by *lamina muscularis mucosae.*

Table 2. Arterial and regional lactate in gut ischemia and reperfusion

|	Baseline	Ischemia	Reperfusion	p
Time(min)	0 minutes	90 minutes	60 minutes	
Total occlusion of SMA				
Luminal microdialysis lactate (mmol/L)				
	0.09(0.06-0.17)	2.37(1.29-2.98)	3.11(2.03-8.06)	0.0001
Gut wall lactate, superficial (mmol/L)				
	1.21(0.78-1.47)	2.17(1.67-3.89)	1.93(1.25-3.51)	0.0011
Gut wall lactate, deeper (mmol/L)				
	0.58(0-0.87)	2.55(1.45-4.97)	1.32(0.12-2.69)	0.0001
Mesenteric venous lactate (mmol/L)				
	0.78(0.56-1.25)	5.64(3.53-7.80)	2.95(1.67-5.21)	0.0002
Arterial plasma lactate concentration (mmol/L)				
	0.74(0.55-1.12)	1.66(0.86-7.06)	2.85(1.77-3.79)	0.0002
Partial occlusion of SMA				
Luminal microdialysis lactate (mmol/L)				
	0.09(0.06-0.51)	1.66(0.07-3.97)	0.97(0.06-2.4)	0.006
Gut wall lactate, superficial (mmol/L)				
	1.08(0.99-1.26)	2.56(1.61-3.42)	1.48(0.62-2.58)	0.0004
Gut wall lactate, deeper (mmol/L)				
	0.75(0.06-1.09)	1.79(0.04-2.75)	0.71(0.10-1.51)	0.006
Mesenteric venous lactate (mmol/L)				
	0.77(0.56-1.55)	2.71(0.62-5.03)	1.26(0.13-3.27)	0.027
Arterial plasma lactate concentration (mmol/L)				
	0.76(0.63-1.22)	1.20(0.56-2.33)	1.09(0.60-5.25)	0.27
Control				
Luminal microdialysis lactate (mmol/L)				
	0.10(0.03-0.16)	0.08(0.02-0.22)	0.06(0.01-0.22)	0.15
Gut wall lactate, superficial (mmol/L)				
	1.00(0.87-1.13)	0.88(0.84-1.14)	0.83(0.73-1.28)	0.30
Gut wall lactate, deeper (mmol/L)				
	0.80(0.72-0.91)	0.65(0.45-0.79)	0.69(0.44-1.04)	0.34
Mesenteric venous lactate (mmol/L)				
	0.78(0.76-1.23)	0.66(0.59-0.80)	0.60(0.46-1.13)	0.02
Arterial plasma lactate concentration (mmol/L)				
	0.69(0.61-1.00)	0.70(0.54-0.92)	0.72(0.51-1.53)	0.79

Statistical significance of changes within the group was tested with Friedmans (the last column) test.

5.2.2 Systemic versus selective regional blood flow reduction

5.2.2.1 Blood flow and oxygen transport

Superior mesenteric artery blood flow decreased in parallel with decreasing aortic blood flow during moderate and severe cardiac tamponade. The proportional superior mesenteric arterial blood flow from aortic blood flow remained fairly constant. Both systemic and regional oxygen delivery decreased gradually during progressive cardiac tamponade. Systemic oxygen consumption increased transiently during moderate tamponade and decreased during severe tamponade. Mesenteric oxygen consumption remained constant during moderate tamponade with concomitantly increasing mesenteric oxygen extraction ratio. Only at the end of the experiment did oxygen consumption became supply dependent (see original publication III for details).

Regional mesenteric oxygen extraction ratio increased from 0.22 (0.13-0.44) at baseline to 0.56 (0.31-0.73) after selective blood flow reduction by occlusion of superior mesenteric artery and remained elevated (p=0.006). In the pericardial tamponade model mesenteric oxygen extraction ratio was already higher at baseline 42(41-52)%. Oxygen extraction ratio increased to 87(80-89)% at the end of the experiment.

5.2.2.2 Gut luminal lactate and jejunal mucosal arterial pCO_2 gradient

Jejunal mucosal to arterial pCO_2 gradient tended to increase before jejunal luminal microdialysate lactate in both selective gradual decrease of superior mesenteric arterial blood flow and during gradual decrease of systemic blood flow by cardiac tamponade (Figure 7). Jejunal luminal lactate release remained lower during low systemic blood flow induced by pericardial tamponade as compared to selective gradual reduction of superior mesenteric arterial blood flow (Figure 7). Superior mesenteric arterial blood flow was 3(1-5) ml/kg/min during sustained low blood flow in selective ischemia and comparably, 3(2-5) ml/kg/min during the first step of cardiac tamponade and

even lower, 1.5(1.5-3) ml/kg/min during the severe tamponade. Concomitantly, systemic arterial lactate concentrations were 1(0.6-1.5) mmol/L in sustained low superior mesenteric arterial blood flow (60 minutes) in selective gut ischemia and 6(5-7) mmol/L and 11(11-12) mmol/L during the two steps of systemic low blood flow states.
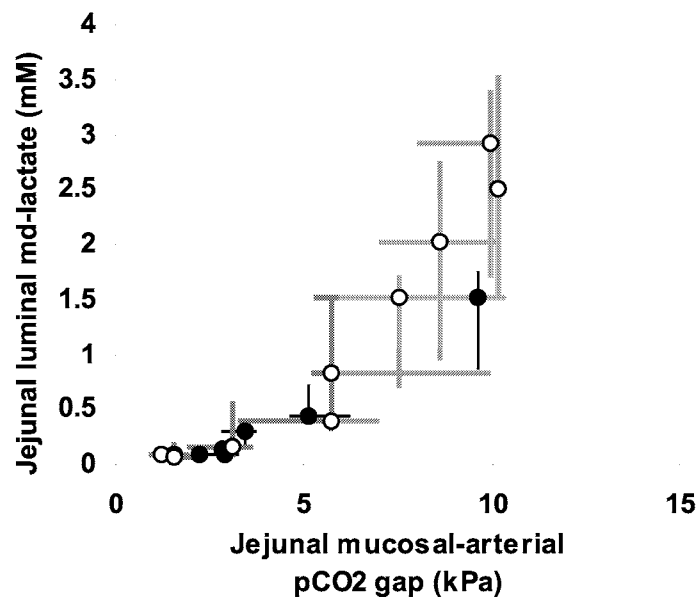
Figure 7. Jejunal mucosal to arterial pCO_2 gradient in relation to corresponding jejunal luminal microdialysate lactate in selective gradual occlusion of superior mesenteric arterial flow (open circles) and gradual decrease of systemic blood flow by cardiac tamponade (black circles). Each data point represents median with interquartile ranges of both pCO_2 gradients and luminal lactate concentrations.

5.2.2.3 Metabolic changes

Jejunal luminal microdialysate lactate increased 60 minutes after reduction of aortic flow (Figure 8, $p<0.05$). Peak concentration of jejunal luminal microdialysate lactate was 1.5 (0.9-1.7) mmol/L at the end of the experiment. Increased luminal lactate could be largely explained by arterial hyperlactatemia in severe pericardial tamponade as illustrated in the figure 9, where the upper normal limit of gut luminal lactate in relation to arterial lactate is applied.
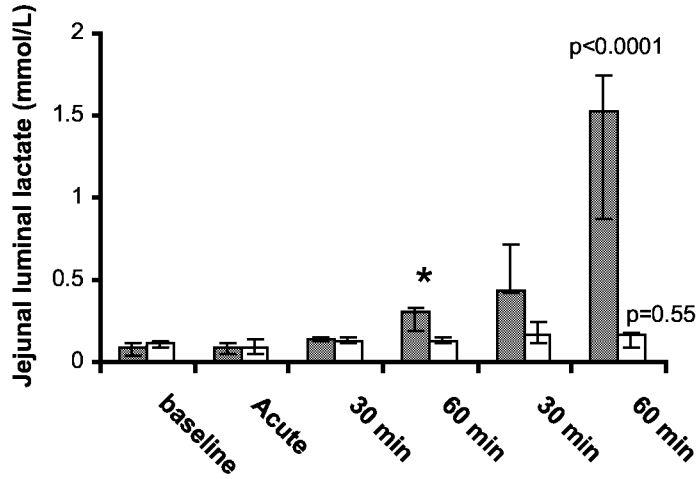
Figure 8. Jejunal luminal lactate in progressive pericardial tamponade (gray columns) and control animals (white columns). Data is presented as median (quartiles). P-values refer to Friedman and * $p<0.05$ by Wilcoxon's signed rank test (within group comparison to baseline)
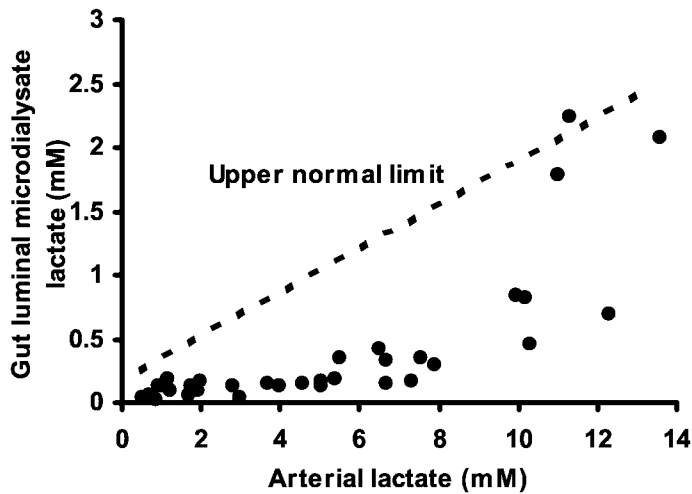
Figure 9. Individual jejunal luminal microdialysate lactate concentrations (black circles) plotted against corresponding arterial lactate concentrations. Dashed line represents the upper normal limit of gut luminal lactate in relation to arterial lactate concentration.

Jejunal lactate production was 1.0(0.7-2.6) μmol/kg/min and 1.8(0.8-3.5) μmol/kg/min at baseline conditions in pericardial tamponade and regional selective ischemia models, respectively. Selective occlusive ischemia with sustained low superior mesenteric arterial blood flow (and decreased VO_2) induced a mesenteric (jejunal) lactate production increase to 10(5-14) μmol/kg/min. Progressive pericardial tamponade induced a transient uptake of lactate followed by a tendency towards increased lactate production and finally a return to baseline levels (p=0.029).

Mesenteric venous lactate to pyruvate ratio increased concomitantly with decreasing mesenteric oxygen supply in pericardial tamponade. In parallel, we observed increasing jejunal pyruvate uptake as estimated by increasingly negative mesenteric pyruvate exchange from -100(-150-320) μmol/kg/min at baseline to –380(-470- -290) μmol/kg/min and –210(-300- -100) μmol/kg/min after first and second step of tamponade (p=0.026).

5.2.3 Distributive shock and comparison to cardiogenic shock

5.2.3.1 Blood flow and oxygen transport

The acute response to endotoxin infusion was an abrupt reduction of hepatic arterial flow only followed by a profound systemic hypoperfusion. The fractional celiac trunc flow (from aortic flow) decreased from basal 28 (21-40)% to 25 (17-38)% (p=0.046) before systemic blood flow decreased. Celiac trunk flow reduction paralleled with decrease in fractional hepatic arterial flow (from aortic flow) from 8 (4-11)% to 2 (1-4)% (p=0.028) before further decrease in systemic blood flow. Hepatic arterial blood flow behaved as an isolated unit also within celiac circulation; fraction of hepatic arterial flow from celiac trunc decreased from 23 (13-50)% to 5 (3-28)% (p=0.028) before further decrease in trunc flow. Contrasting the above mentioned, superior mesenteric arterial blood flow followed the changes in the aortic flow. The fraction of Qsma at baseline was 28 (20-44)% and was maintained at 29 (20-44)% at the time of the first reduction of hepatic arterial flow (p=0.92).

Disregarding the difference in baseline fractional blood flows, celiac trunk blood flow appeared to decrease to a disproportionately low level during hypodynamic endotoxin shock and remain lower during hyperdynamic shock as compared to primary increase during gradually decreasing systemic blood flow. Superior mesenteric arterial blood flow changed in proportion to systemic blood flow both during endotoxin shock and low systemic blood flow by pericardial tamponade (Figure 10). Splanchnic oxygen supply decreased but the apparent splanchnic oxygen consumption remained constant with increasing extraction ratio from median 37% to 75%. Colonic oxygen delivery decreased during the hypodynamic shock and oxygen extraction increased with moderate increase in oxygen consumption. Interestingly, with increasing oxygen delivery during hyperdynamic phase of the shock, colonic oxygen consumption first decreased indicating oxygen extraction problem.
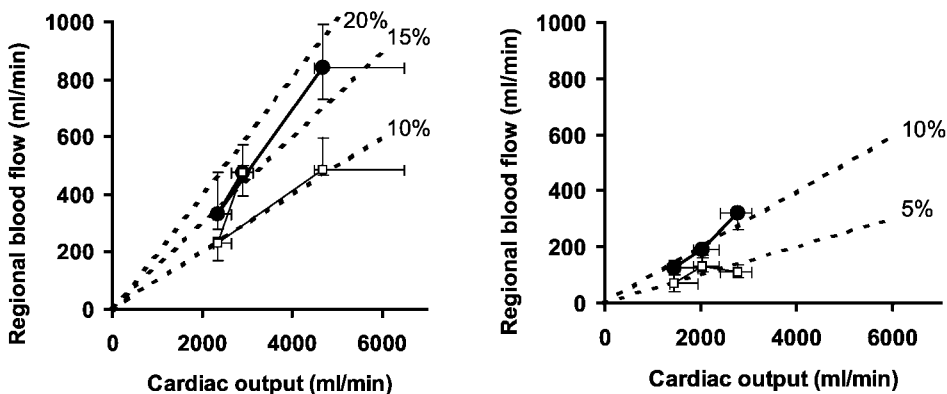
Figure 10. Regional blood flows, superior mesenteric arterial blood flow (filled circles) and celiac trunk blood flow (open quadrangles) in relation to cardiac output in endotoxin shock (left panel) and low systemic blood flow induced by pericardial tamponade. Dashed lines represent the fractions from cardiac output as indicated by numeral values.

5.2.3.2 Metabolic changes

Gastric and colonic mucosal to arterial pCO_2 gradients increased during hypodynamic endotoxin shock. Gastric mucosal to arterial pCO_2 gradient increased again during hyperdynamic shock while colonic pCO_2 gradient

decreased to baseline levels. No major increase occurred in jejunal mucosal to arterial pCO_2 gradient over the study period (Table 3). Mucosal to end-tidal pCO_2 gradient overestimated mucosal to arterial pCO_2 gradient. Bland-Altman analysis showed greater bias and poorer precision in endotoxemic as compared to the control animals. Bias and precision in the control animals were 0.9 kPa and 0.9 kPa, and 2.0 kPa and 2.2 kPa in the endotoxic animals, respectively. Intestinal luminal microdialysate lactate concentration increased at the end of the experiment only in colon in the endotoxin group from low close to 0 concentrations to 0.4(0.1-0.8) mmol/L and 0.5(0.2-1.3) mmol/L during the last hour of the experiment (p<0.05), while no colonic luminal lactate release occurred in time controls. Luminal lactate in jejunum and stomach remained unchanged (Figure 11). When intestinal luminal lactate release (concentrations every 30 minutes) and mucosal end-tidal pCO_2 gradients over the study period were compared, no obvious association between the two variables was seen.

Table 3. Mucosal arterial pCO2 gradients in endotoxin shock (n=7) and controls (n=6). P-values refer to nonparametric analysis of variance for repeated measurements (Friedman).

	Baseline	2h	4h	12h	p (0-4h)	p (4-12h)
Gastric arterial pCO2 gradient (kPa)						
ETX	2.5(2.2-3.3)	5.7(5.1-6.7)	3.8(3.0-4.8)	8.6(7.2-9.4)	0.002	0.104
Control	2.1(1.8-2.2)	2.6(1.9-3.7)	2.1(1.8-2.4)	2.4(2.0-3.0)	0.513	0.938
Jejunal arterial pCO2 gradient (kPa)						
ETX	2.0(1.8-2.3)	3.3(3.1-4.5)	2.6(2.4-3.7)	2.8(2.5-4.5)	0.004	0.865
Control	2.1(1.3-3.1)	2.0(1.7-3.3)	2.2(1.4-2.7)	2.4(1.5-3.5)	0.957	0.209
Colonic arterial pCO2 gradient (kPa)						
ETX	3.8(3.1-4.1)	6.2(5.5-7.1)	6.5(5.4-8.3)	3.6(3.6-7.8)	0.009	0.054
Control	2.2(1.4-3.0)	2.4(1.6-2.8)	2.0(1.6-3.0)	3.1(2.0-3.5)	0.676	0.355

Abbreviations: ETX; endotoxin group, pCO2; partail tension of carbon dioxide.
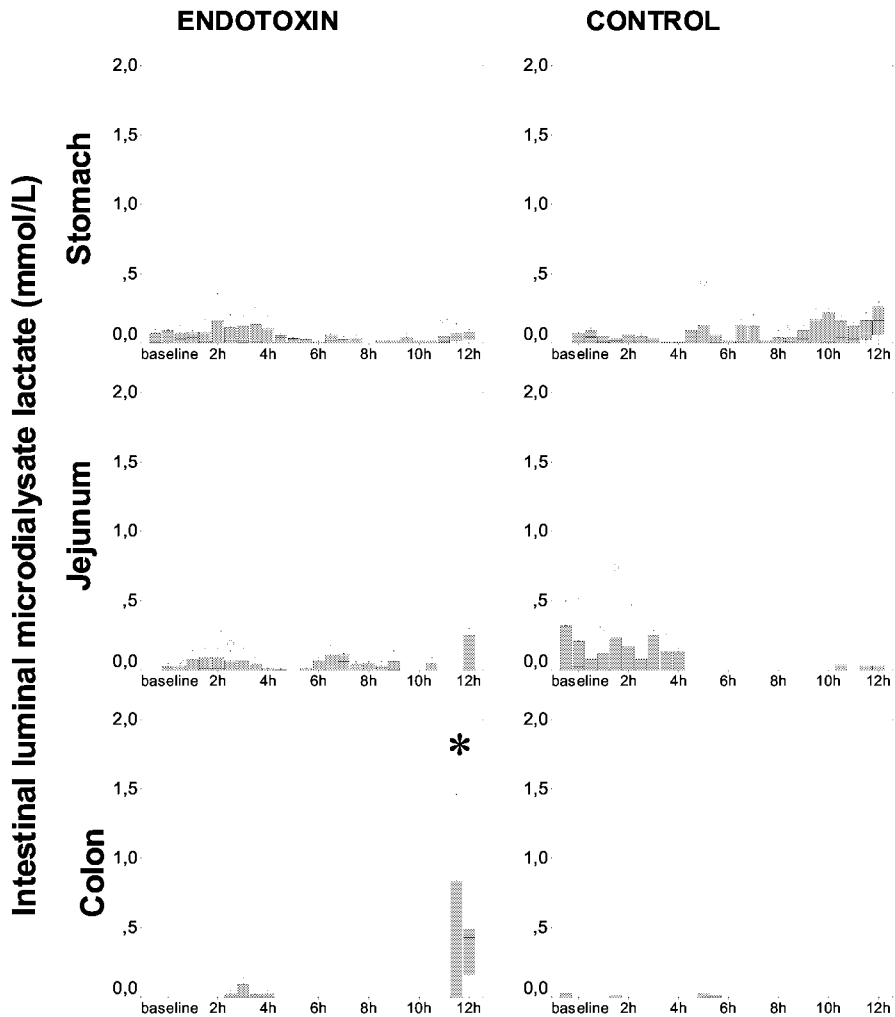
ENDOTOXIN   CONTROL

Figure 11. Intestinal luminal microdialysate lactate measured every 30 minutes from stomach, jejunum and colon during baseline conditions and the two phases of endotoxin shock (left side) and control conditions (right side) Data is presented as median and inter quartile range. * p<0.05.

Gastric lactate exchange turned negative from baseline 0(0-1)µmol/kg/min to −2(-2- -1)µmol/kg/min after 6 hours of endotoxemia and increased to 2(1-

3)µmol/kg/min (p<0.05) after hyperdynamic shock was established. Jejunal lactate exchange decreased from 6(5-7)µmol/kg/min to 2(0-4)µmol/kg/min after 6 hours and returned to 7(1-8)µmol/kg/min (p<0.05). Colonic lactate exchange decreased from 6(5-7) µmol/kg/min to 2(0-4) µmol/kg/min after 6 hours of endotoxin infusion and increased back to 7(1-8) (p<0.05) µmol/kg/min at the end of the experiment. Ketone body ratio increased only in arterial samples during the acute primary response to endotoxin infusion from 1.3(0.9-1.5) to 2.5(1.9-3.1) (p=0.008).

Mucosal-arterial pCO_2 gradients appeared to be associated only with CO_2 content gradient in hypodynamic endotoxin shock but not during hyperdynamic shock. Instead, increasing pCO_2 gradient was associated with increasing veno-arterial CO_2 content gradient in progressive pericardial tamponade (Figure 12).
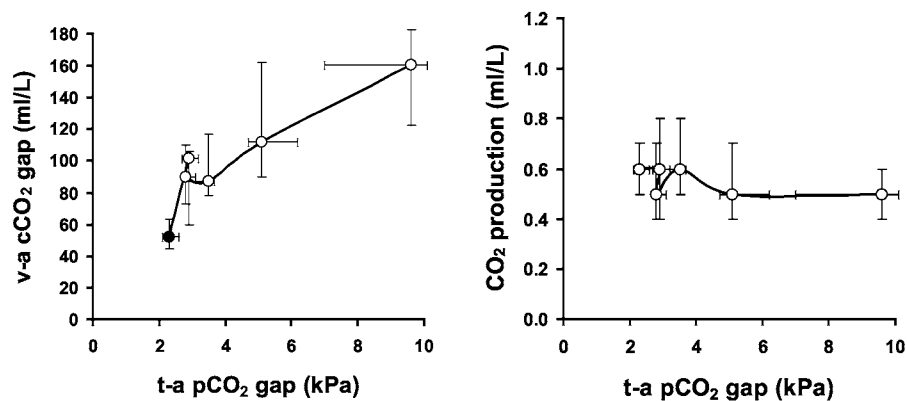
Figure 12. Jejunal mucosal-arterial pCO_2 gradients (t-a pCO_2 gap) in relation to jejunal veno-arterial CO_2 content gradients (v-a cCO_2 gap, left panel) and regional (mesenteric) CO_2 production (right panel) during baseline (black circle) and gradual pericardial tamponade every 30 minutes (open circles). Data is presented as median (interquartile range).

5.2.4 Histology

Histological grading according to Chiu [157] revealed no injury in the subepithelial mucosal in either jejunum or colon (Table 4). Further analysis focusing on mucosal epithelial integrity revealed cryptal epithelial injury in colon while jejunal mucosal epithelium remained intact (Figures 13 and 14).
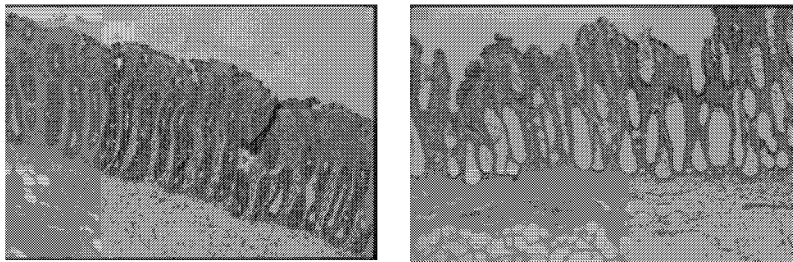
Figure 13. Representative picture of colonic mucosal histology in one control animal after the experiment (left) and after the endotoxin challenge (right). Note the different appearance of crypts and destroyed Goblet cells after endotoxin.
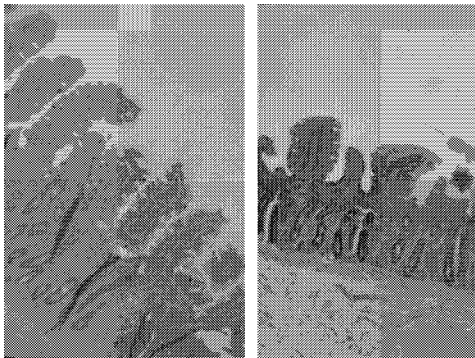
Figure 14. Representative picture of jejunal mucosal histology in one control animal after the experiment (left) and after the endotoxin challenge (right).

Table 4. Histological analysis of colon and jejunum in endotoxin shock (n=5) and controls (n=6).

	Egr		Chiu		Villi/Cryptae		Epithelium	
Jejunum	baseline	12h	baseline	12h	baseline	12h	baseline	12h
ETX	2(2-2)	1(1-1)	0(0-0)	0(0-0)	1(0.3-1.8)	2(1-2)	0.5(0-1)	1(1-2)
Controls	2(1-2)	1.5(1-2)	0(0-0)	0(0-0)	1(0.8-1.3)	1(1-1)	1(0.8-1)	0.5(0-1)
Colon								
ETX	1(1-1)	1(1-1)	0(0-1.5)	0(0-0)	1(0.3-1)	3(3-3)*#	1(0.3-1)	2(2-3)*#
Controls	1(0.8-1)	1(1-1)	0(0-0)	0(0-0)	0(0-1)	1(1-1.8)	1(0.8-1)	1(1-1.8)

Egr; amount of eosinophilic granulocytes (0-3), Chiu; subepithelial injury according to Chiu (0-5)
Villi/cryptae; integrity of the structure in villus (jejunum) or crypts (colon) (0-3). Epithelium; Integrity
of the mucosal epithelial cells (0-3).* refers to p< 0.05, Wilcoxon. # refers to p<0.05, Mann-Whitney U.

6   DISCUSSION

6.1   Methodological aspects

6.1.1   Animal models

Large animals were used to enable us to some extent extrapolate the findings to human anatomy and physiology. Additionally, this enabled us to use clinically applicable equipment of large sizes. We used animal models with barbiturate-based anesthesia. This may have an effect on the splanchnic circulation as suggested by others [161]. Each study was, however, accomplished according to a randomized, controlled protocol. Therefore the effect of other treatment modalities than actual intervention equals between different groups. Even though we aimed at a standardised, randomized, controlled experimental setting, the major limitation in our study was small number of animals and large inter-individual variation and therefore the results have to be interpreted accordingly.

For studying endotoxin shock we used an experimental model of normovolemic, hyperdynamic, hypotensive, normoglycemic, prolonged endotoxin shock in anesthetized pigs. The model was adopted and modified from Santak et al [162]. The duration of 12 hours was sufficient to produce clinically relevant state of circulation with high cardiac output and low blood pressure and vascular resistance. Since we adjusted the endotoxin dosage according to pulmonary arterial pressure, the amount of endotoxin varied immensely. This was expected since the animals were farm animals of random breed. Thereby the microbial environment or microbial exposure was not standardized. We used a continuous infusion of endotoxin, which can be regarded to produce a more clinically model of endotoxin shock when compared to a single bolus of endotoxin used in many previous studies on endotoxicosis [163].

6.1.2 Intestinal luminal microdialysis

Intestinal luminal microdialysis has not been used earlier to evaluate the intestinal mucosal metabolism. The main finding of the first experiment (I) in this series of investigations was the consistent early increase of intestinal luminal microdialysate lactate both in partial and total occlusion of superior mesenteric artery. Systemic non-ischemic hyperlactatemia induces intestinal luminal lactate release only at very high arterial lactate levels and the effect of arterial hyperlactatemia on luminal lactate can be estimated, as we have done. In contrast, high intestinal wall microdialysate lactate can be achieved without regional intestinal ischemia, as demonstrated by nonischemic lactate clamp (lactate infusion).

Since local intestinal perfusion deficits may remain uncovered even with maintained global parameters [24, 65], it is of major importance to aim at local tissue specific monitoring. In our experiment gut luminal microdialysis revealed intestinal hypoperfusion, when systemic signs of inadequate perfusion were still absent and arterial lactate levels normal or marginally increased.

Lactate concentrations in the luminal microdialysate cannot be directly extrapolated to absolute luminal concentrations of lactate. In particular, the lactate analysis of ours necessitated the use of high infusion rate (2 µl/min) of dialysate in order to gain adequate sample volumes. On the other hand, the semipermeable membrane used in the capillaries was highly permeable with cut-off limit as high as 60000 Daltons. Thereby good recovery of lactate within the blood stream and high concentrations of lactate observed during gut ischemia suggest a marked increase in luminal lactate during hypoperfusion. In addition in vitro recovery of the capillaries with the above mentioned flow rate was high (70-80%). In order to perform quantitative luminal lactate measurements, in vivo calibration, described by Lönnroth et al [164], should be used. Since this requires adding known concentrations of the substrate of interest to the dialysate and zero point of substrate flux extrapolation by linear

regression, this is not feasible in the non-steady state conditions such as an acute ischemia-reperfusion.

Speculatively, there are at least four factors which contribute to gut luminal lactate concentration: 1) mucosal epithelial release of lactate, 2) diffusion of lactate from subepithelial mucosa and capillaries and 3) epithelial cellular uptake of lactate, 4) bacterial content of intestine may an additional confounding variable to gut luminal lactate measurement since in fecal mass L-lactate is converted to D-lactate by different bacterial species and further to short-chain fatty acids [165]. In contrast, bacterial release of lactate does not interfere with the luminal lactate measurement since bacteriae produce D-lactate instead of L-lactate. The method for lactate detection in this series of experiments was L-lactate specific.

6.1.3 Intestinal tonometry

We used both saline tonometry and semiautomatic gas analyzer [159] with tonometer catheters for detection of mucosal pCO_2 in our experiments. Saline tonometry can now be regarded as early version of methodology with long stabilization periods and possibilities to erroneous measurements [166]. H2-blockers were not used since the benefit is not clearly shown [167, 168, 169], even though gastric mucosal acid generation may interfere [170]. One of the findings in publication II was variation in threshold blood flow leading to luminal lactate release. In addition, mucosal arterial pCO_2 associated to increasing luminal lactate varied both in selective ischemia and cardiogenic shock. This indicates individual variation in how severely reduced perfusion defect has to be in order to induce anaerobic metabolism. Schlichtig and Bowles suggest that distinguishing aerobic and anaerobic CO_2 production is possible by dissociation of mucosal pCO_2 from regional pCO_2 [129]. This seems appealing but can be regarded only applicable in laboratory conditions. The same group later studied the relation between lactate and CO_2 accumulation in an in vitro model of intestinal ischemia/dysoxia [171]. They concluded that one of the fundamental acid-base changes in intestinal dysoxia is lactate accumulation. Their data indicate that in a bulk intestinal

preparate CO_2 accumulation is accompanied by lactate accumulation, but also that aerobic CO_2 accumulation precedes anaerobic CO_2 production. Lactate production may be increased during nonischemic conditions as well due to enzymatic changes [172, 173]. In both selective and systemic progressive intestinal ischemia models our results are in accordance to the above mentioned: aerobic CO_2 production (or only stagnation) precede gut luminal lactate release. Consequently, simultaneous detection of intestinal mucosal pCO_2 and luminal lactate seems feasible in detecting intestinal mucosal epithelial dysoxia in low flow states and possibly in endotoxin/septic shock.

6.1.4 Comparison between intestinal pCO_2 gradients and luminal lactate

An increase in intestinal mucosal to arterial pCO_2 gradient is fundamental physiology and indicates low blood flow and CO_2 stagnation locally. In the present series of experiments, restoring blood flow rapidly induced decrease in mucosal to arterial pCO_2 gradients. Luminal lactate, on the other hand, increased only after pCO_2 gradients, possibly indicating that widening pCO_2 gradient reflected a blood flow reduction that was severe enough to cause mucosal dysoxia. After restoring the blood flow luminal lactate remained high or even increased. This may indicate that luminal lactate is at least in part blood flow independent indicator of metabolic changes in intestinal mucosa. In endotoxin shock the setting may be quite different. Firstly, flow redistribution may occur within splanchnic area as, indeed, appears according to the present experiment of endotoxin shock. Secondly, blood flow redistribution may occur within a single organ, as it occurs in intestinal wall. Thirdly, metabolic changes in addition to mere changes in blood flow contribute to CO_2 exchange. Finally, pCO_2 as compared to CO_2 content gradients or regional CO_2 production may give a wrong impression on tissue versus systemic CO_2 gradients.

Luminal lactate release in colon during endotoxin shock may indicate mucosal dysoxia leading to epithelial injury as we suggest. This may, however be an oversimplification. Endotoxemia may induce aerobic glycolysis [174]

and inhibits pyruvate dehydrogenase activity [175]. In addition hyperinsulinemia induces aerobic lactate production [176]. In the present endotoxin shock experiment we saw a need of increasing infusion rates of glucose to maintain blood glucose levels. This may indirectly indicate a hyperinsulinemic state. Alternatively hepatic gluconeogenesis may have been impaired as occurs in newborn rats [177]. A far more common phenomenon, however, is insulin resistance during endotoxemia [178]. Hyperinsulinemia in endotoxemia occurs already after 30 minutes after starting endotoxin infusion in minipigs [179]. Since colonic luminal lactate release occurred only in the very end of the experimental model of endotoxin shock, we believe it does represent dysoxia rather than altered aerobic lactate release related to insulin-induced lactate release.

6.2 Comparison of selective intestinal ischemia and systemic blood flow reduction

6.2.1 Blood flow, oxygen extraction and metabolic changes

Oxygen extraction ratio, however, increased much higher in systemic low blood flow state indicating broader range of physiological buffering in comparison to selective ischemia. In addition, gut luminal lactate release was more profound in selective intestinal ischemia as compared to systemic blood flow reduction and increasing luminal lactate during systemic low blood flow could be explained to a large extent by systemic hyperlactatemia alone. Instead, with comparably low regional blood flow gut mucosal to arterial pCO_2 gradients increased similarly indicating equally severe flow reduction. If regional blood flow decreases mucosal arterial pCO_2 gradient increases with constant metabolic rate. Increased gradient per se does not allow judgment on aerobic versus anaerobic metabolism in individual animals. PCO_2 gradient measurement does not allow the detection of gut mucosal ischemia, but is merely a complex result of metabolic and perfusion conditions. Therefore mucosal arterial pCO_2 gradient may be sensitive for regional blood flow changes but gut luminal lactate is specific for changes in local mucosal

epithelial metabolism. As to what extent the changes are uniform in the different segments of intestine remains speculative. Based on the present study of endotoxin shock it appears that metabolic adaptation/changes are not uniform in different segments. This is in accordance with experiments by others [180].

Mesenteric (jejunal) lactate exchange (production) increased after gradual selective blood flow reduction of superior mesenteric artery. This was not a uniform phenomenon during low systemic blood flow. Also, mesenteric venous sampling represents a pooled, flow dependent concentration of lactate in the veins draining from different layers of gut wall. Decreasing the whole gut perfusion may lead to redistribution of perfusion within the wall, as occurs in sepsis [112] and regional hypotension [181] where blood flow is redistributed in favor of mucosa. In addition, the local vasodilatory autoregulation counteracting e.g. sympathetic vasoconstriction is stronger in mucosa than muscular layer of gut wall [45]. Under conditions of supply dependent oxygen consumption, reflex vasoconstriction redistributes blood flow towards mucosa instead of muscular layer [182]. Hence, it is likely, that lactate gradient over mesenteric vascular bed does not represent metabolic state of mucosal epithelial cells. Instead, it is reasonable to believe that gut luminal lactate by microdialysis capillary compressed against mucosal epithelium represents metabolic changes specifically in mucosal epithelial cells.

Systemic blood flow reduction is tolerated comparably well by intestinal mucosal epithelium. This is supported by high regional oxygen extraction ratio and relatively low gut luminal lactate release during low systemic blood flow. The apparent lack of jejunal mucosal hypoxia during pericardial tamponade may be related to the neurohumoral compensatory mechanisms which alter the distribution of capillary blood flow between different layers of intestinal wall [15, 181, 183].

6.3 Comparison of distributive and cardiogenic shock

6.3.1 Blood flow, oxygen transport and metabolic changes

Superior mesenteric arterial and celiac trunk blood flows decreased in proportion to decreasing systemic blood flow during progressive pericardial tamponade. Preferential reduction of visceral blood flow in low oxygen supply states is proposed to be one of the key mechanisms of inadequate gut perfusion in different disease states [12, 184, 185, 186, 187]. This was not observed in the present study. These findings are in accordance with several recent studies [27, 28, 29]. Prevention of hypovolemia by controlled fluid therapy is one likely factor contributing to the well preserved fractional visceral blood flow in the present study.

In contrast, endotoxin shock induced redistribution of regional blood flow within splanchnic region as evidenced by disproportionately low celiac trunk blood flow during both hypo- and hyperdynamic shock. At the same time superior mesenteric arterial blood flow was largely maintained. In addition, the acute primary response to endotoxin infusion was abrupt decrease of celiac trunk and in particular hepatic arterial blood flow only followed by decreasing aortic (systemic) blood flow. Superior mesenteric arterial blood flow decreased in parallel with aortic blood flow during the primary response.

The increase in mesenteric venous ketone body ratio suggests that the cellular redox status in the gut as a whole was impaired during low systemic blood flow (cardiogenic shock), even when luminal lactate suggests absence of mucosal epithelial dysoxia. Surprisingly, in endotoxin shock (distributive shock) only arterial ketone body ratio increased during the acute primary response to endotoxin, which may indicate that lung is one of the primary targets of endotoxemia. Indeed, the primary isolated hepatic arterial blood flow reduction was uniformly followed by a sudden, profound increase of pulmonary arterial pressures and right-sided heart failure. Also, endotoxin induced acute lung injury characterised by low PaO_2/FiO_2 ratio and increased fraction of dead space ventilation.

Contrary to what we found in cardiogenic shock, mucosal to systemic (tonometric-end-tidal) pCO_2 gradients were not associated with increasing luminal lactate in endotoxin shock. Mucosal to arterial pCO_2 gradients were associated with veno-arterial CO_2 content gradients only in a low blood flow model and in the hypodynamic phase of endotoxin shock. This implies that the interpretation of pCO_2 gradients is complex. Other investigators have evaluated the small intestinal [188], intraperitoneal [189], sigmoid [189] or bladder [78,190] versus arterial pCO_2 gradients detection as indicators of total splanchnic or systemic perfusion. We suggest that none can be taken *per se* as a surrogate for total splanchnic perfusion, nor do they indicate when perfusion deficiency in relation to metabolic demand was profound enough to induce ischemia/dysoxia locally.

PCO_2 gradient changes over the three regions did not reflect the adequacy of total splanchnic perfusion over the whole study period of endotoxin shock. Thus, evaluation of adequacy of perfusion in the whole splanchnic bed from one location is impossible. Instead luminal lactate release in colon was associated with local mucosal epithelial injury as verified by histology.

6.4 Summary

A new microdialysis capillary has been validated for lactate measurement in the present series of experiments. Recovery rate of the capillary is high both in vivo and in vitro. The capillary is especially made for concomitant measurement of intestinal mucosal partial tension of CO_2 by tonometry. It offers a possibility to gain valuable additional information of the metabolic state of intestinal mucosal epithelial cells. Indeed, intestinal luminal lactate detection enables researchers, and later clinicians, to detect a change in intestinal mucosal epithelial lactate metabolism or even the onset of anaerobic metabolism for the first time. Recognizing the limitations of the current clinically available methods, it appears that intestinal microdialysis has potential for clinical application after further studies both in laboratory and clinical setting.

Referring to the specific aims of the present series of experiments, it is concluded that selective occlusive mesenteric ischemia induces gut luminal lactate release which can be detected early by gut luminal microdialysis. Gradual selective mesenteric blood flow reduction reveals the immense variation in individual response to blood flow reduction emphasizing the importance of individual monitoring and need to avoid arbitrary assumptions of thresholds on severity of blood flow reduction.

Regional mesenteric blood flow is by far maintained to the same extent as systemic blood flow in cardiogenic shock. Gut luminal lactate release occurs reasonably late during prolonged severe cardiogenic shock indicating that jejunal mucosal epithelial cells may have a strong ability for down regulation of metabolism.

In contrast endotoxin shock induces both acutely and during the two, hypo- and hyperdynamic, phases of distributive shock, as by definition, redistribution of blood flow *within* splanchnic vascular bed. Therefore surrogate markers of perfusion over one visceral region may only coincidentally reflect tissue perfusion over another part of the region. Therefore, multi-location monitoring is encouraged, but also multi-modular monitoring enabling the evaluation of not only perfusion, but also the metabolic state of the organ of interest.

Colonic mucosal lactate release is increased during hypotensive hyperdynamic distributive shock. This lactate release is associated with histopathological changes in colonic mucosal epithelial cells. Therefore luminal lactate detection may have potential for clinical monitoring of the vitality of intestinal mucosal epithelial cells not only in low blood flow states (cardiogenic shock) but also in septic/hyperdynamic state of blood circulation (distributive shock).

In summary, redistribution of regional blood flow may occur within the visceral circulation even without drug interventions. Therefore the surrogate markers of perfusion over one visceral region do not reflect changes over whole splanchnic vascular bed. Recognizing the importance of visceral circulation [19, 191], there is a need for regional or even local metabolic monitoring in parallel with evaluating the adequacy of perfusion. Intestinal

luminal lactate measurement by microdialysis offers such additional information based on which the state of mucosal epithelium can be characterized.

7   CONCLUSIONS

- A new microdialysis capillary with high recovery was validated for intestinal lactate detection
- Intestinal mucosal epithelial metabolism can be monitored by luminal microdialysis
- Gut luminal lactate release occurs reasonably late in cardiogenic shock as compared to selective intestinal ischemia indicating adaptive mechanisms during low systemic blood flow
- Distributive shock induces by definition redistribution of blood flow also within splanchnic region and therefore surrogate markers of perfusion over one region do not reflect perfusion conditions in another region
- Intestinal luminal lactate release is associated with mucosal epithelial injury. Therefore it has potential for metabolic monitoring of intestinal mucosal epithelial cells

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